# Supplementary Materials

# Evaluation of 4-Aminoquinoline Hydrazone Analogues as Potential Leads for Drug-Resistant Malaria

**Rachael N. Magwaza [1,2], Muna Abubaker [2], Buthaina Hussain [3], Michael Haley [4], Kevin Couper [4], Sally Freeman [1,*] and Niroshini J. Nirmalan [2,*]**

[1] Division of Pharmacy and Optometry, School of Health Sciences, University of Manchester, Manchester M13 9PT, UK
[2] School of Science, Engineering and Environment, University of Salford, Manchester M5 4WT, UK; r.n.magwaza@salford.ac.uk (R.N.M.); m.abubaker2@salford.ac.uk (M.A.)
[3] Faculty of Pharmacy, Al-Zaytoonah University of Jordan, Amman 17138, Jordan; buthina.hussein@zuj.edu.jo
[4] School of Biological Sciences, Lydia Becker Institute of Immunology and Infection, University of Manchester, Manchester M13 9PT, UK; michael.haley@manchester.ac.uk (M.H.); kevin.couper@manchester.ac.uk (K.C.)
* Correspondence: sally.freeman@manchester.ac.uk (S.F.); n.j.nirmalan@salford.ac.uk (N.J.N.)

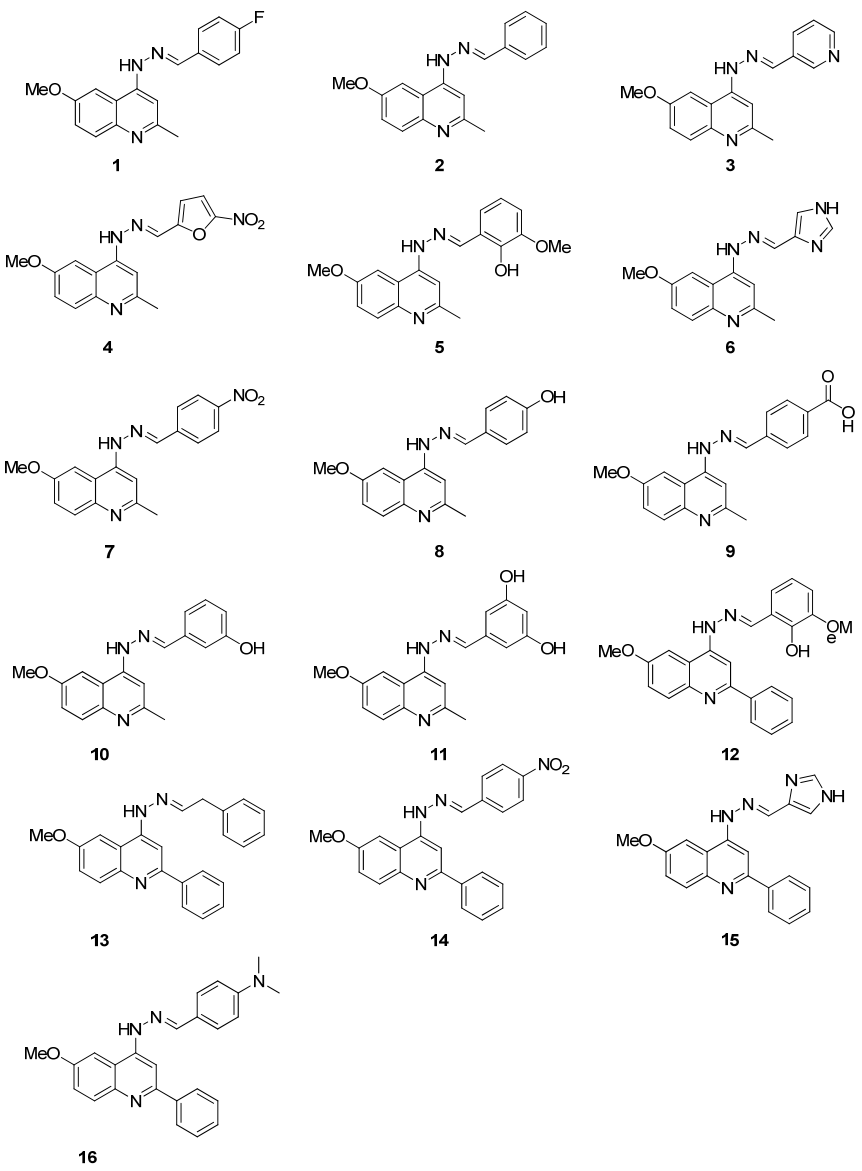
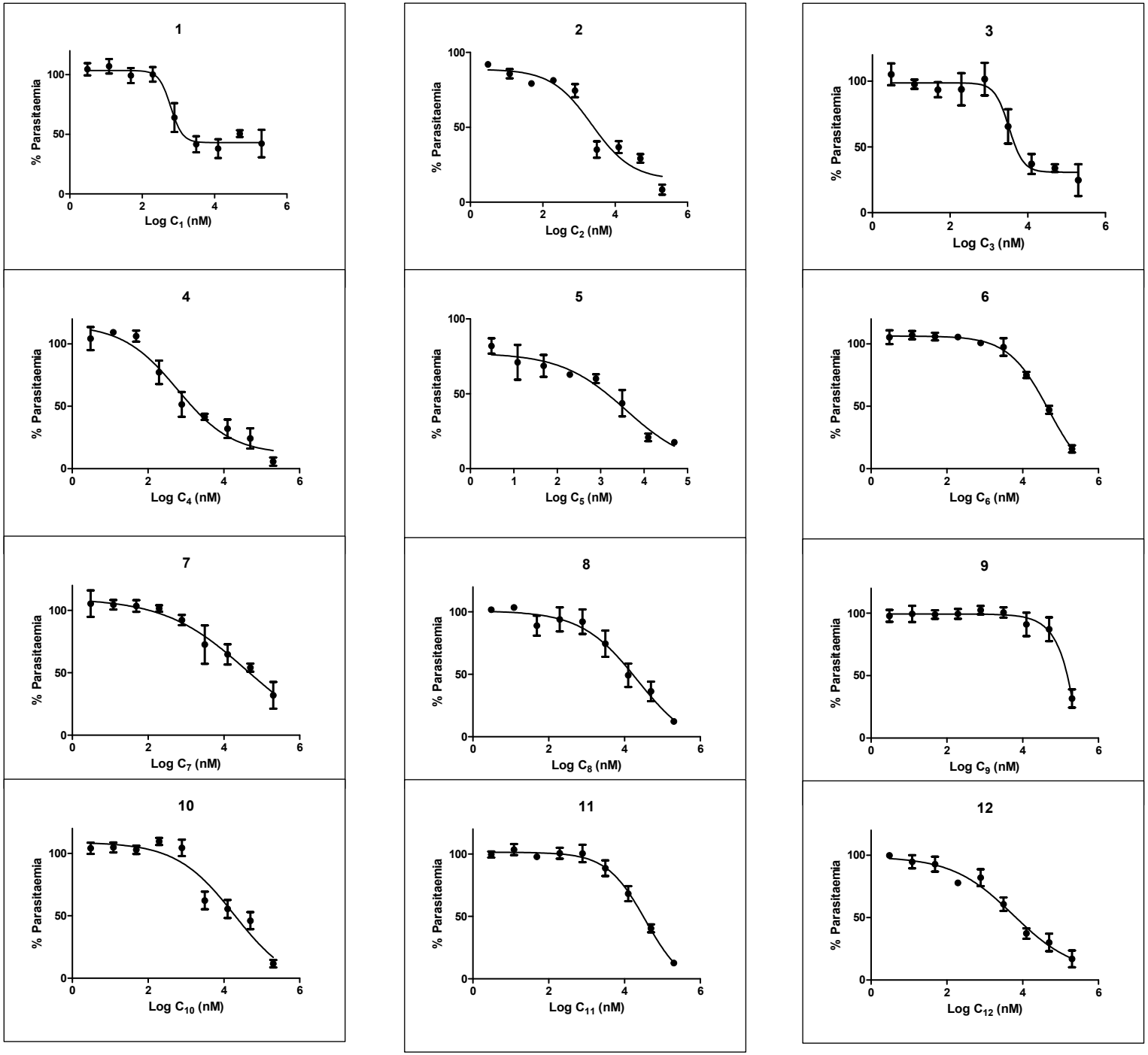



**Figure S1**. Structures of the 4-aminoquinoline hydrazone compounds that were tested for their antimalarial activities.


1
% Parasitaemia
Log C1 (nM)
2
% Parasitaemia
Log C2 (nM)
3
% Parasitaemia
Log C3 (nM)
4
% Parasitaemia
Log C4 (nM)
5
% Parasitaemia
Log C5 (nM)
6
% Parasitaemia
Log C6 (nM)
7
% Parasitaemia
Log C7 (nM)
8
% Parasitaemia
Log C8 (nM)
9
% Parasitaemia
Log C9 (nM)
10
% Parasitaemia
Log C10 (nM)
11
% Parasitaemia
Log C11 (nM)
12
% Parasitaemia
Log C12 (nM)

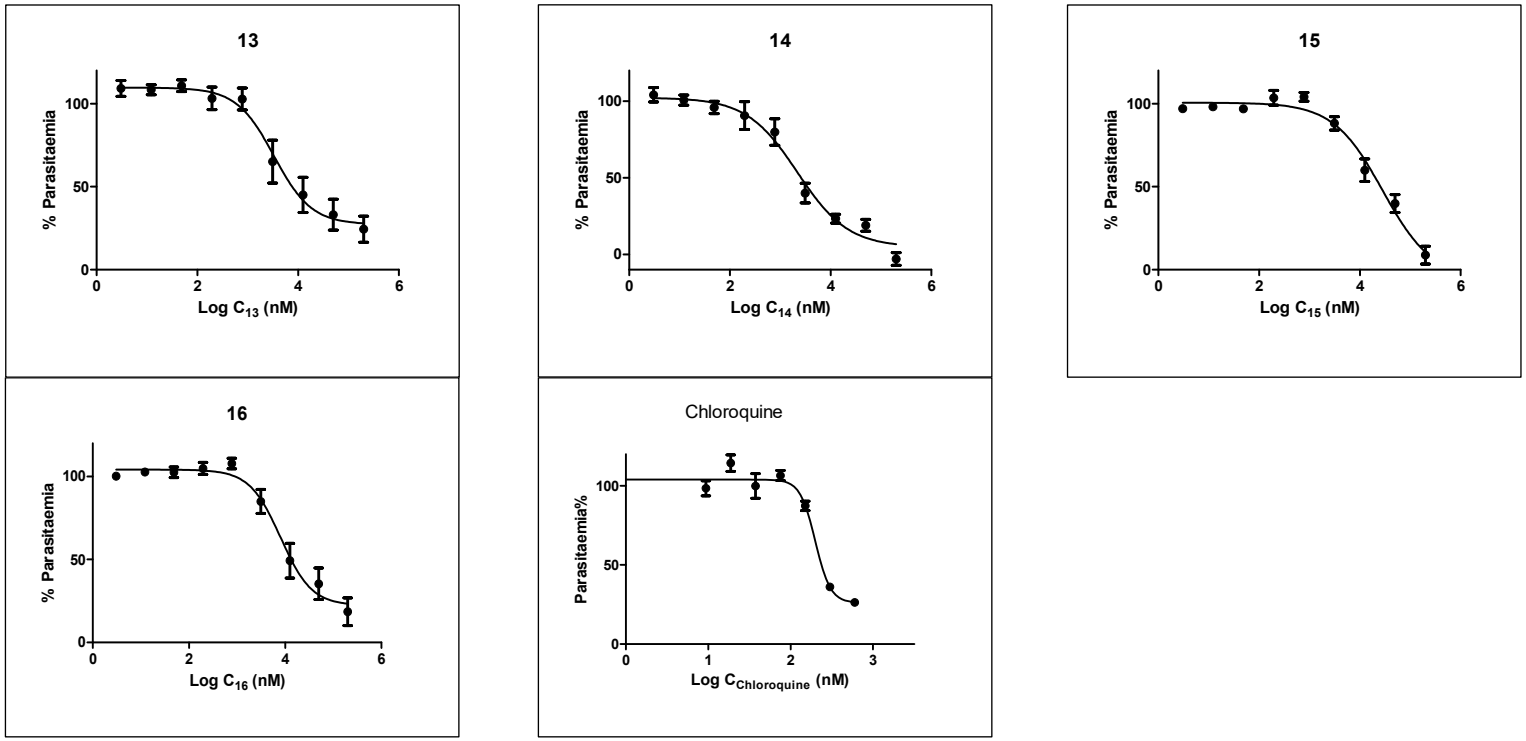



**Figure S2**. The effect of the 4-aminoquinoline hydrazone compounds along with chloroquine tested on the *P. falciparum* K1 strain (trophozoite stage). The 4-aminoquinoline hydrazones were tested in four-fold serial dilutions from 0.00305 - 200 μM, while Chloroquine was tested at a dose of 9.3 - 600 nM. The compounds were incubated for 48 hours and read using SYBER green-based plate reader assay. The experiments were performed three times in triplicates. The data was analysed using GraphPad prism.

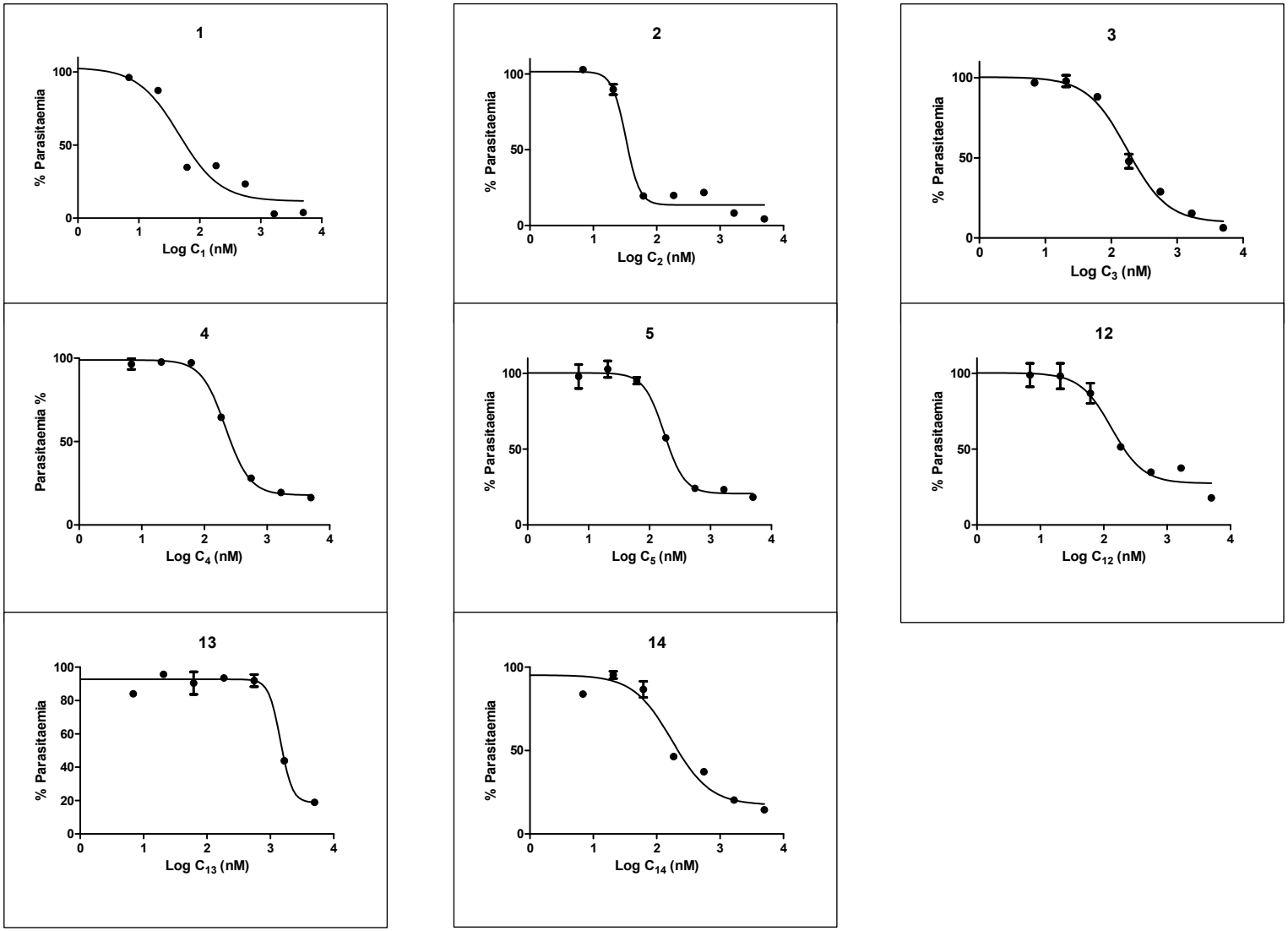
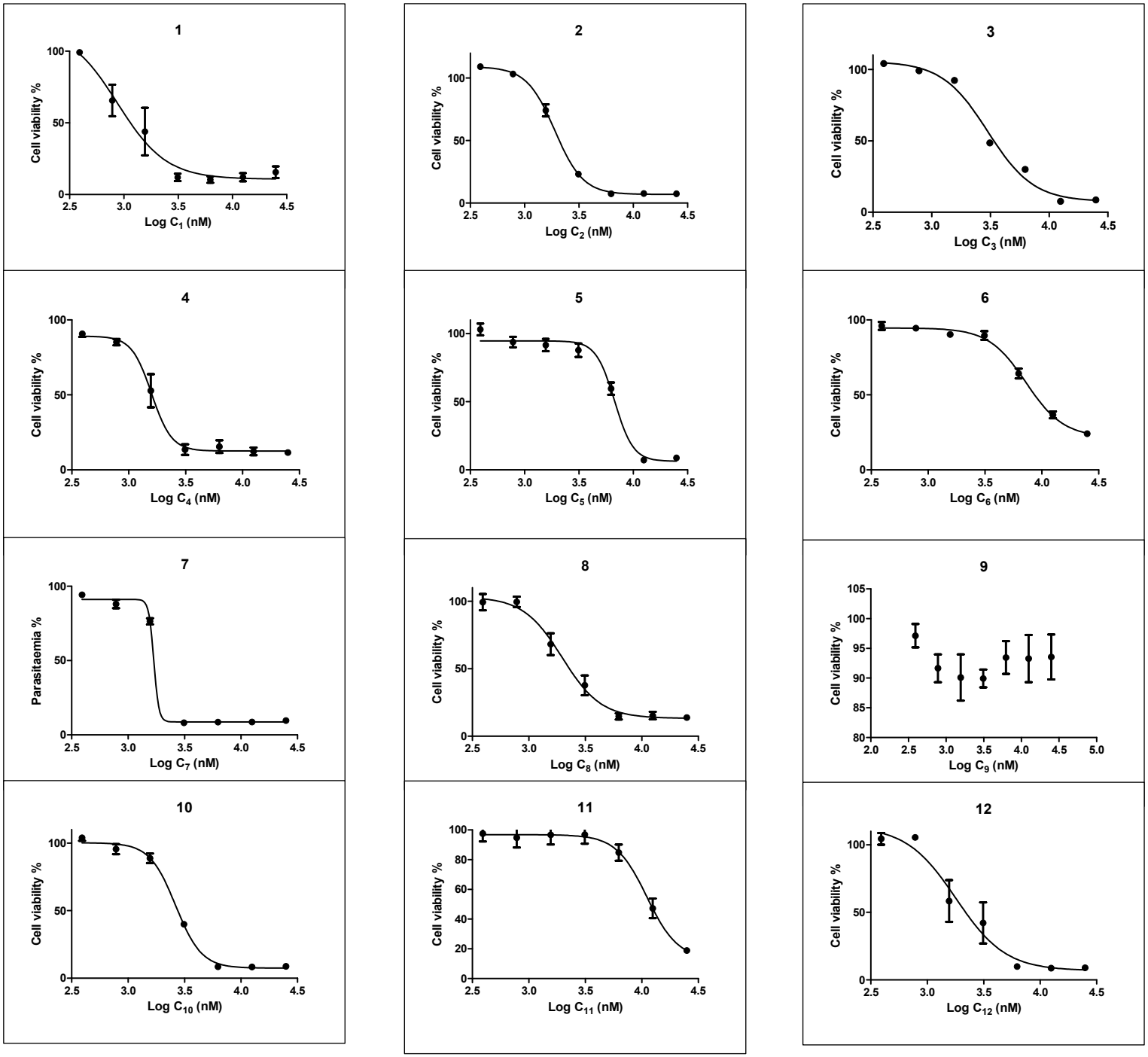



**Figure S3**. The effect dose of the 4-aminoquinoline hydrazone compounds tested on the *P. falciparum* K1 strain (ring stage). The compounds were tested in four-fold serial dilutions from 0.00305 - 200 μM, incubated for 72 hours and read using SYBER green-based plate reader assay. The experiments were performed three times in triplicates. The data was analysed using GraphPad prism.


1
Cell viability %
Log C1 (nM)
2
Cell viability %
Log C2 (nM)
3
Cell viability %
Log C3 (nM)
4
Cell viability %
Log C4 (nM)
5
Cell viability %
Log C5 (nM)
6
Cell viability %
Log C6 (nM)
7
Parasitaemia %
Log C7 (nM)
8
Cell viability %
Log C8 (nM)
9
Cell viability %
Log C9 (nM)
10
Cell viability %
Log C10 (nM)
11
Cell viability %
Log C11 (nM)
12
Cell viability %
Log C12 (nM)

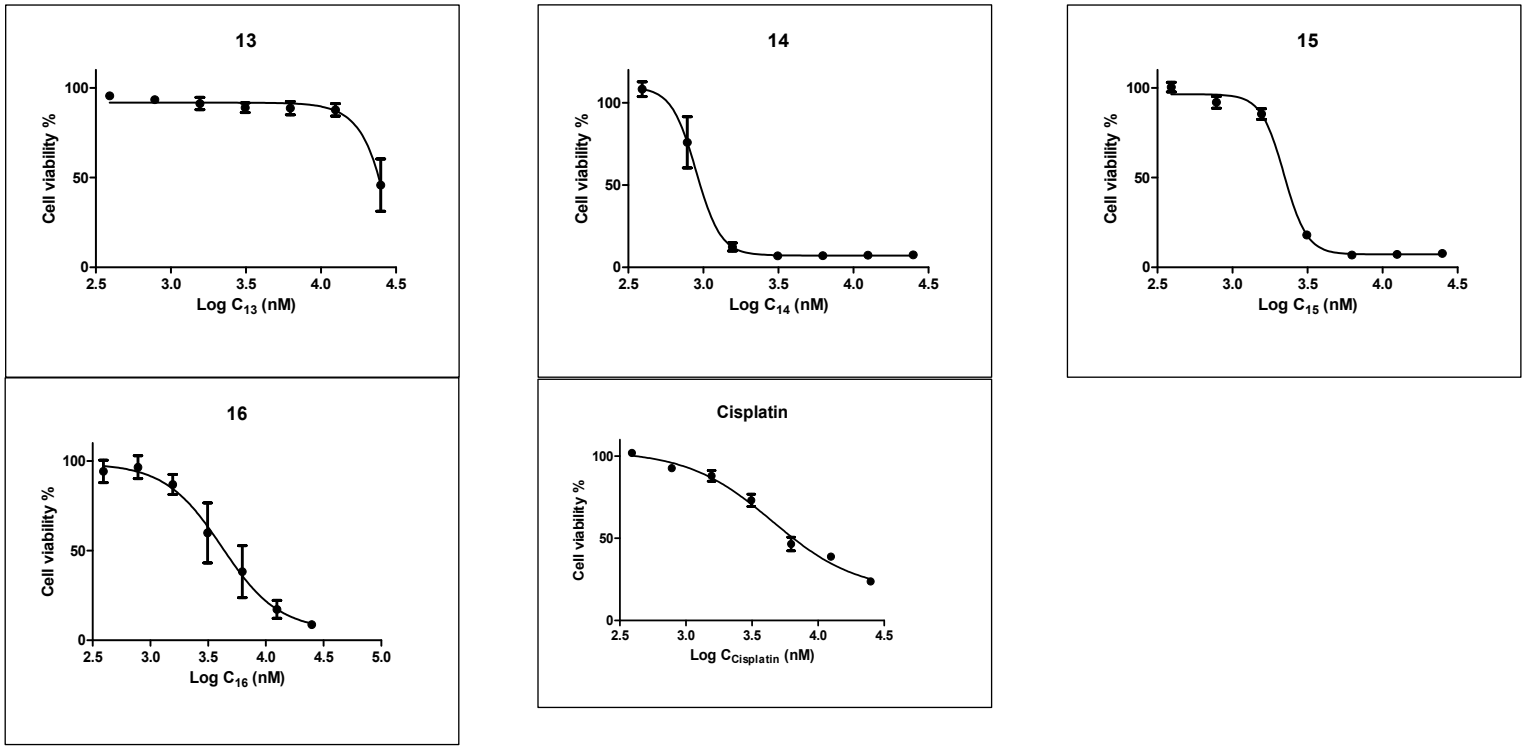
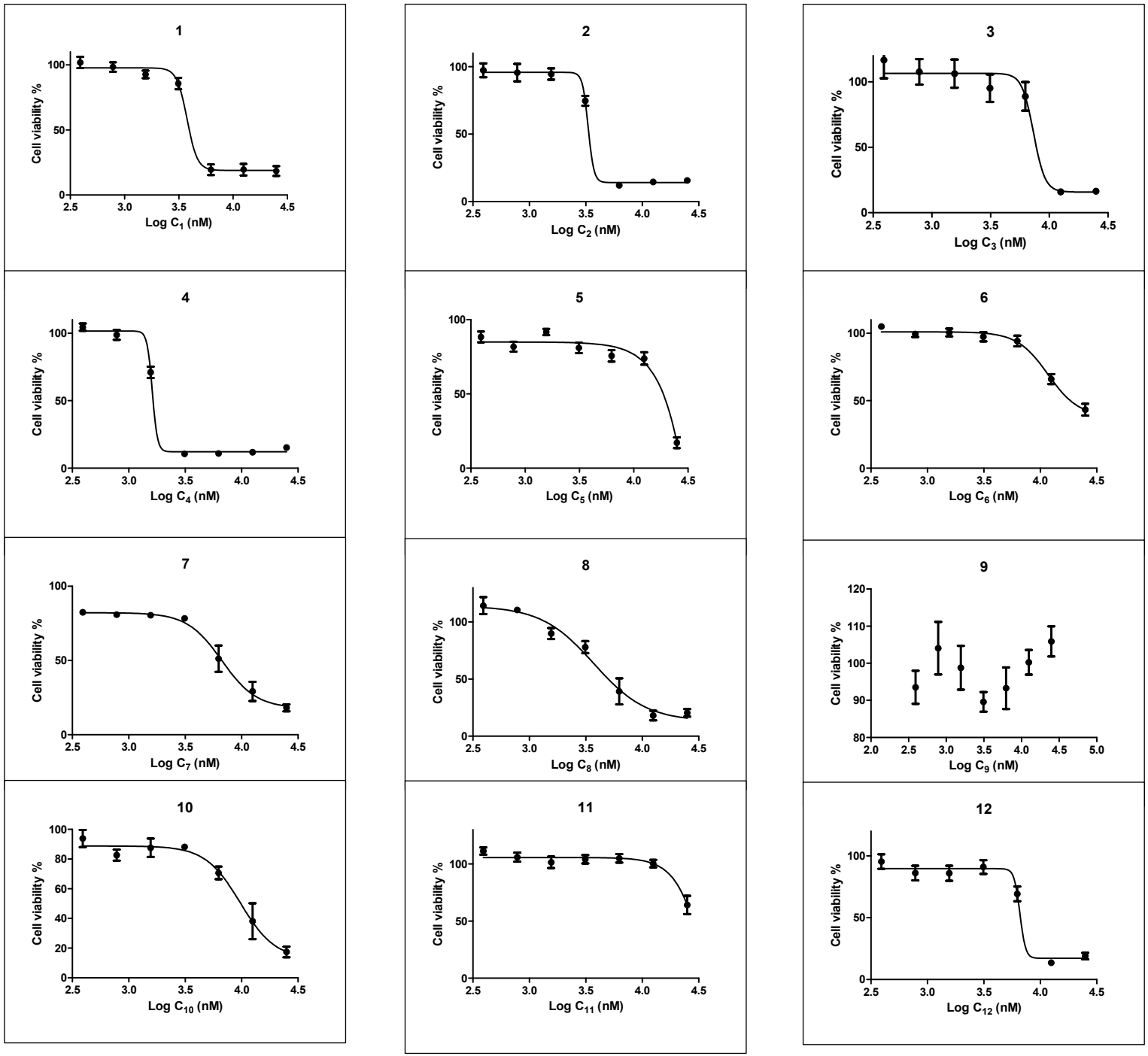



**Figure S4**. MTT assay was assessed on Hep G2 cells. The cells were seeded at 4000 cells per well. The HepG2 cells were incubated with two-fold serial dilution of the compounds at dose range of 0.39 -25 μM for 5 days. Cell viability was determined using the standard MTT assay. Data was analysed using GraphPad prism. The experiments were performed three times; each concentration was repeated three times per experiment.


1
Cell viability %
Log C1 (nM)
2
Cell viability %
Log C2 (nM)
3
Cell viability %
Log C3 (nM)
4
Cell viability %
Log C4 (nM)
5
Cell viability %
Log C5 (nM)
6
Cell viability %
Log C6 (nM)
7
Cell viability %
Log C7 (nM)
8
Cell viability %
Log C8 (nM)
9
Cell viability %
Log C9 (nM)
10
Cell viability %
Log C10 (nM)
11
Cell viability %
Log C11 (nM)
12
Cell viability %
Log C12 (nM)

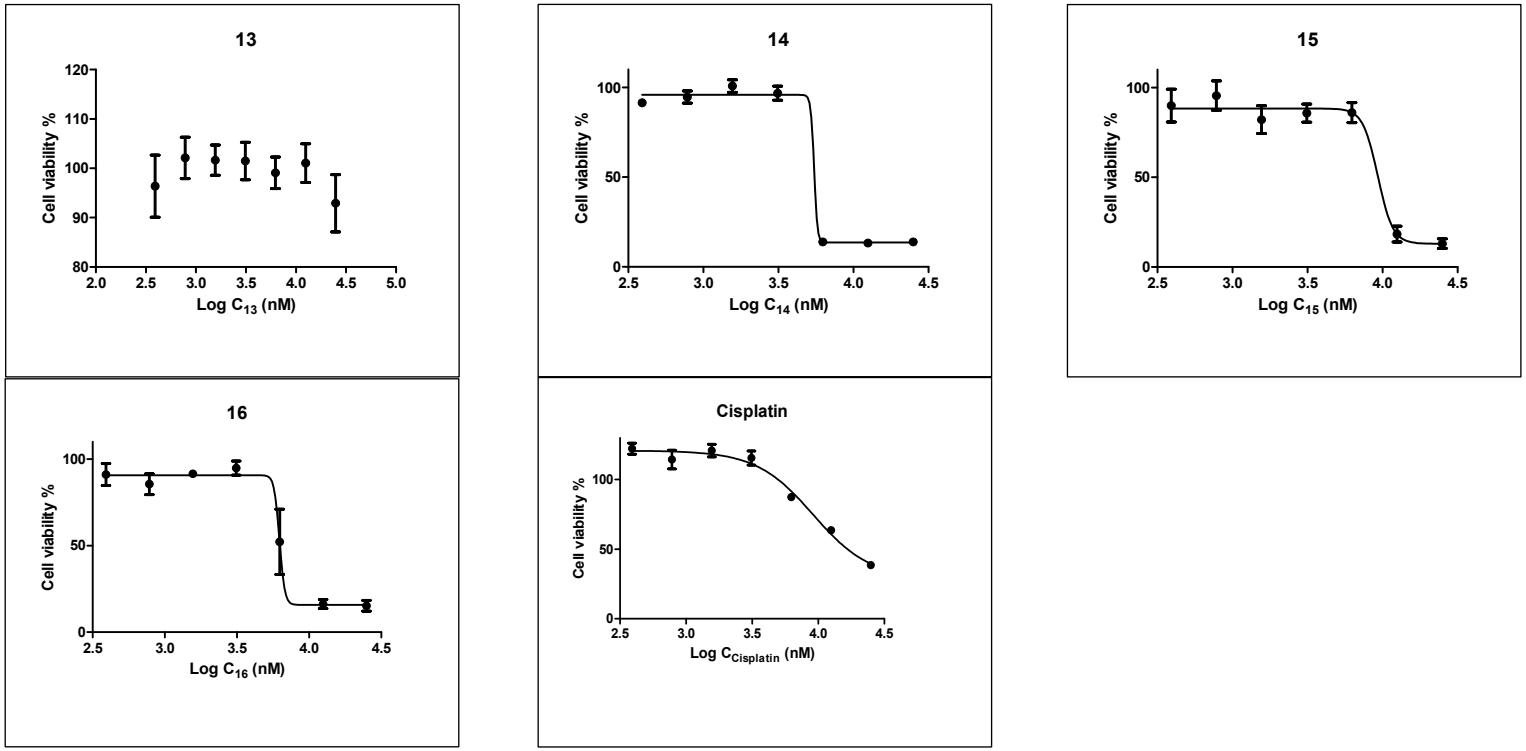



**Figure S5**. MTT assay was assessed on MDBK cells. The cells were seeded at 4000 cells per well. The MDBK cells were incubated with two- old serial dilution of compounds at dose range of 0.39 -25 μM for 5 days. Cell viability was determined using the standard MTT assay. Data was analysed using GraphPad prism. The experiments were performed three times; each concentration was repeated three times per experiment.

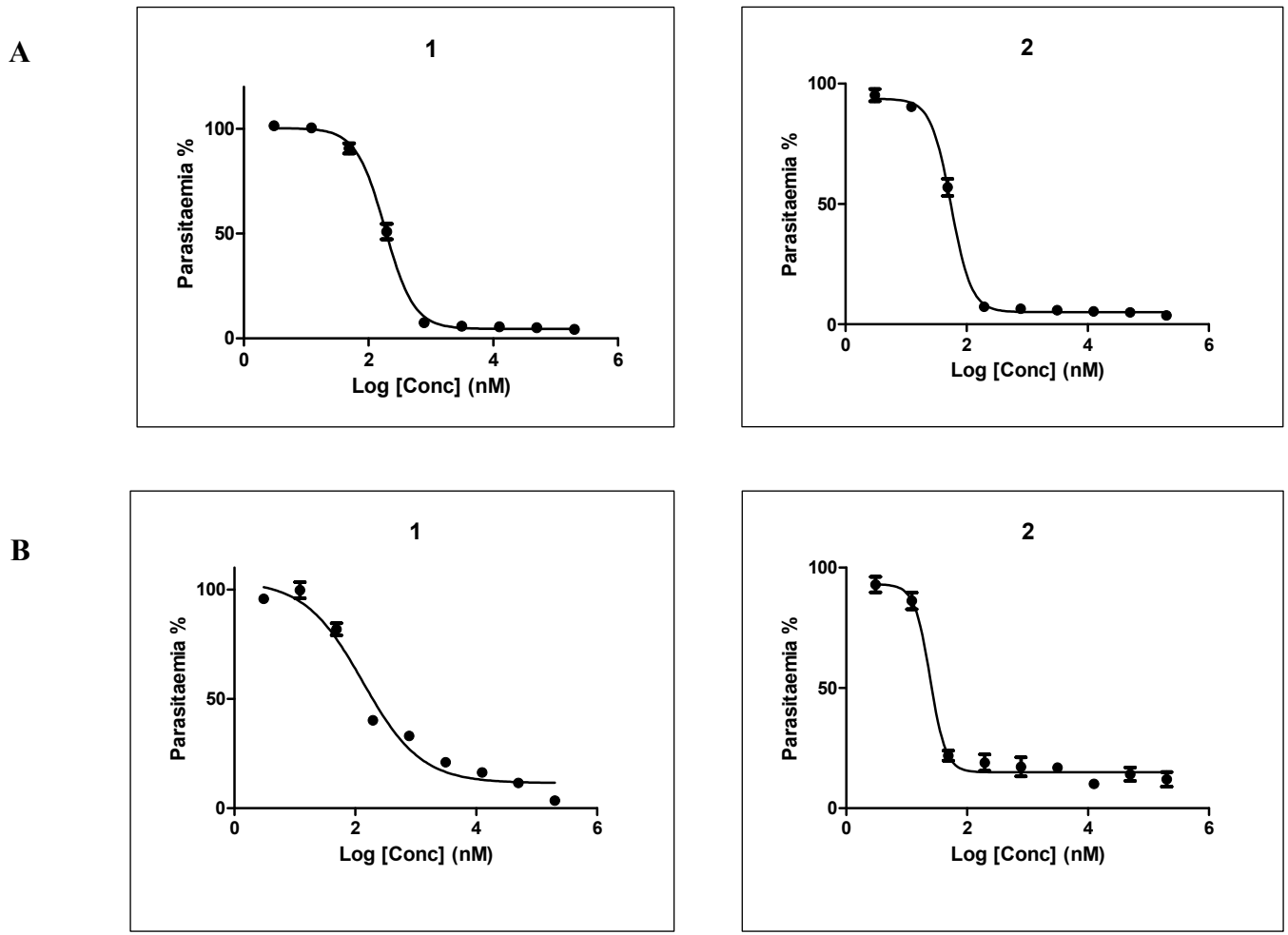



**Figure S6**. The effective dose of the lead 4-aminoquinoline hydrazone compounds **1** & **2** tested on the *P. falciparum* 3D7 A10 (A) and Dd2 (B) strains in a 4-fold serial dilution of dose range from 0.00305 - 200 μM. The compounds were incubated for 72 hours and read using SYBER green-based plate reader assay. The experiments were performed three times in triplicates. The data was analysed using GraphPad prism.

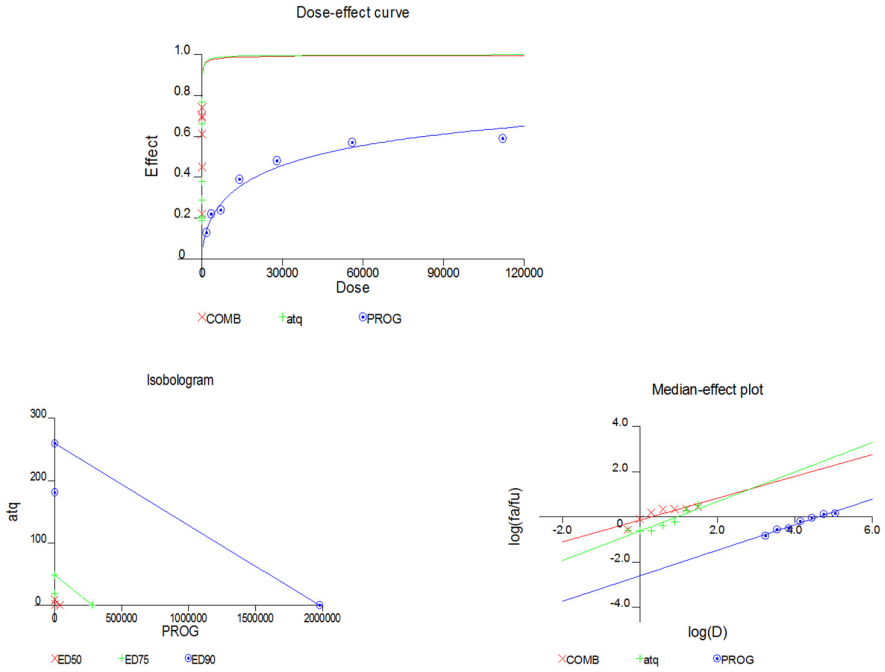
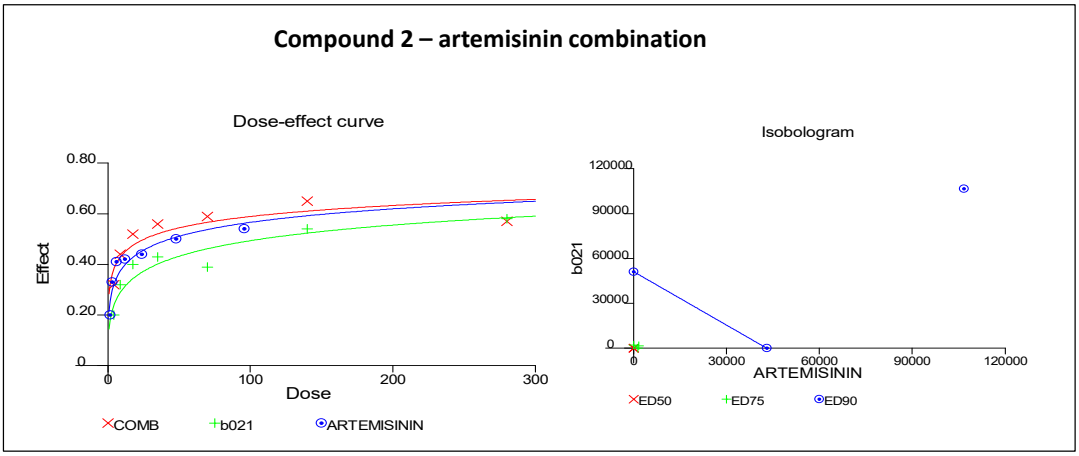
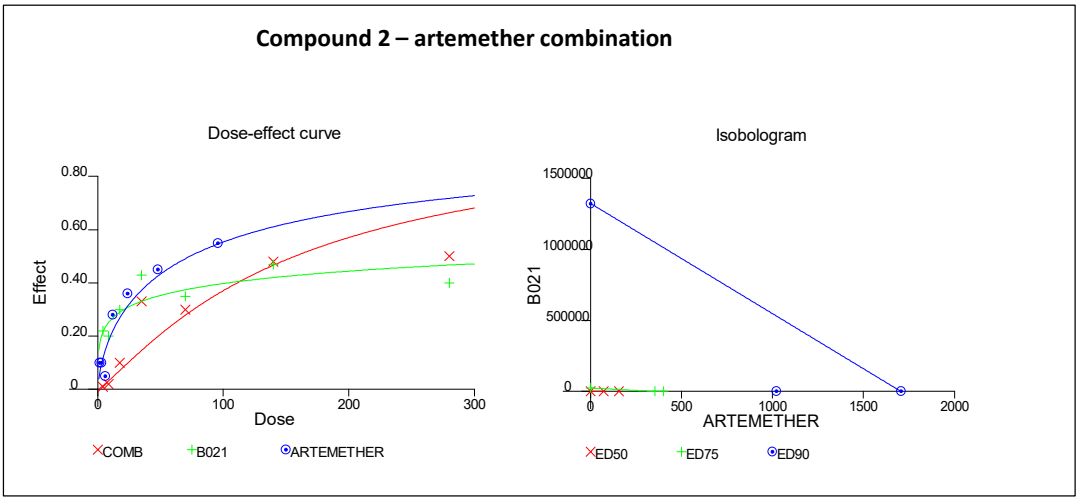



**Figure S7**. Dose effect analysis of atovaquone and proguanil combination on P. falciparum K1 strain. The dose-response data was analysed with CalcuSyn software to give a dose-effect curve and isobologram graph.



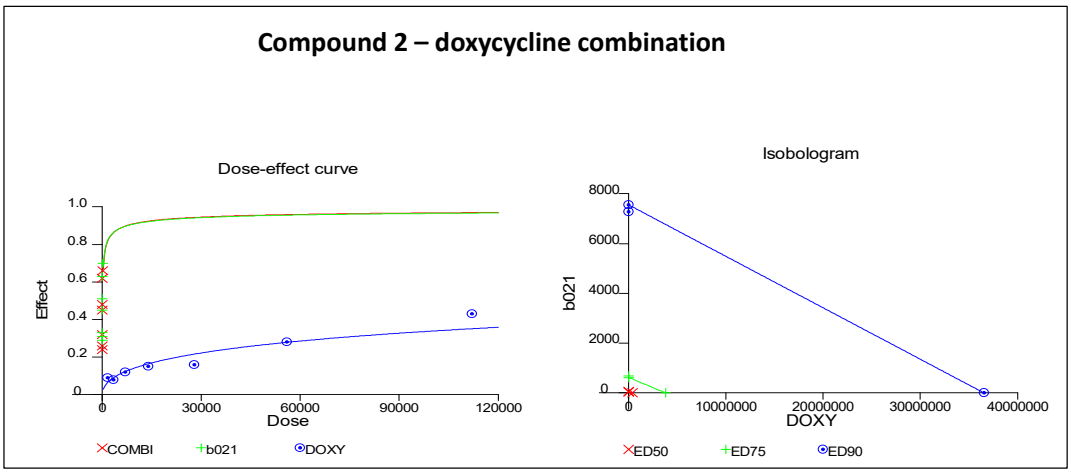




**Figure S8**: Dose effect analysis of Compound **2** and artemisinin, artemether and doxycycline combination on *P. falciparum* K1 strain. The dose-response data was analysed with CalcuSyn software to give dose-effect curve and isobologram graph.