Supporting Information

Identification of Novel Antimalarial Chemotypes via Chemoinformatic Compound Selection Methods for a High Throughput Screening Program against the Novel Malarial Target, PfNDH2 : Increasing Hit Rate Via Virtual Screening Methods

Raman Sharma,[1] Alex. S Lawrenson,[1] Nicholas E. Fisher,[2] Ashley Warman,[2] Alison E. Shone,[2] Alasdair Hill,[2] Alison Mbekeani,[2] Chandrakala Pidathala,[1] Richard K Amewu,[1] Suet Leung,[1] Peter Gibbons,[1] David W. Hong,[1] Paul Stocks,[1] Gemma L. Nixon,[2] James Chadwick,[1] Joanne Shearer,[4] Ian Gowers,[4] David Cronk,[4], Serge P. Parel,[3] Paul M. O'Neill,[1] Stephen A. Ward,[2] Giancarlo Biagini,[2] Neil G. Berry[1*]

[1]Robert Robinson Laboratories, Department of Chemistry, University of Liverpool, Liverpool L69 7ZD UK.

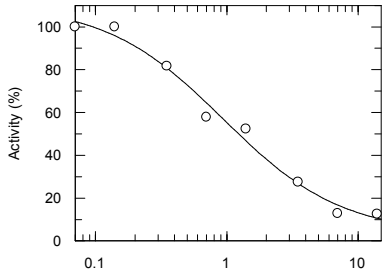
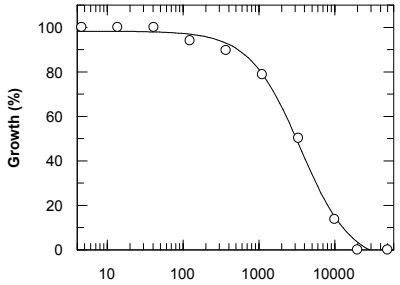
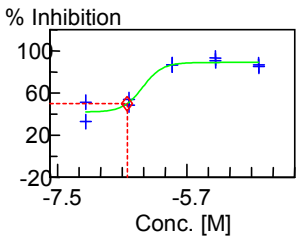
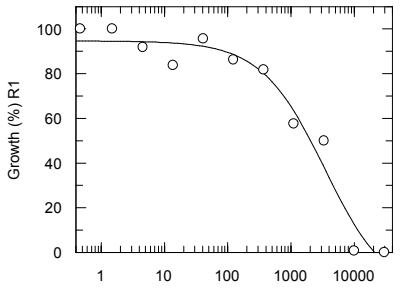
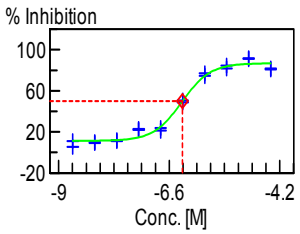
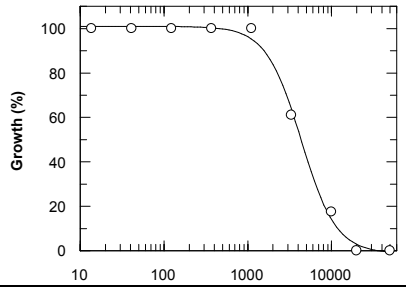
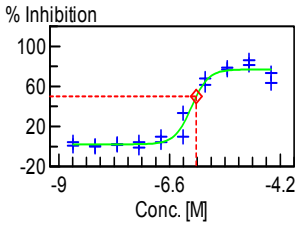
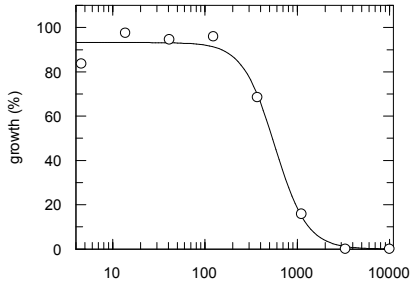
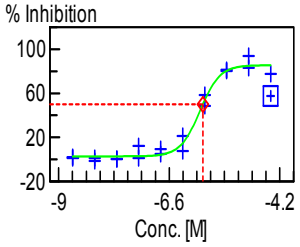
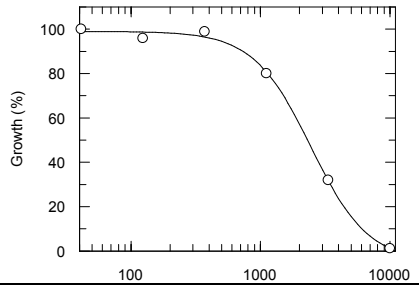
[2]Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK.

[3]BioFocus, Gewerbestrasse, 16, CH-4123, Allschwil, Switzerland.

[4]BioFocus , Chesterford Research Park, CB10 1XL, UK.

*Corresponding author. Tel.: +44 (0)151 794 3877. Fax: 44(0)151 794 3588. E-mail address: ngberry@liv.ac.uk URL: http://www.liv.ac.uk/~ngberry

We provide below sample dose-response curves for the five compounds highlighted in table 4 towards the end of the paper for both PfNDH2 and 3D7 (x axis in nM for 3D7).

	PfNDH2	3D7
1		
2		
3		
4		
5		

Both PfNDH2 and 3D7 dose-response curves were performed at least 3 times. The average IC_{50} values and standard deviations are given in table 4 in the paper.

We have previously examined the effects of detergents (please see Chapter 17 Type II NADH: quinone oxidoreductases of Plasmodium falciparum and Mycobacterium tuberculosis kinetic and high-throughput assays. (Fisher N, Warman AJ, Ward SA, Biagini GA Methods Enzymol. 2009;456:303-20) and appropriate controls were used throughout the screening procedure. For example, control wells used in the calculations did not receive compound (but were diluent controlled) and % inhibition was calculated using wells that had not received CoQ as 100% inhibition. 5 μM HDQ wells were monitored to ensure a high % inhibition was being achieved. In addition KCN was present in the assay buffer in order to control for/prevent non specific/background inhibition. We also checked that "tool" compounds (HDQ for the PfNDH2 screen) had no significant effect on NADH absorbance in the absence of enzyme (no-membrane controls). Using the screening methods the pharmacology of the standard compound HDQ was found to be the expected IC50 value of 100 nM. The HTS however was not designed to differentiate between competitive, non-competitive or uncompetitive inhibition (this is true for the majority of screening methods). However, analyses of key compounds during the subsequent QSAR (publication in preparation) have confirmed that the inhibition of PfNDH2 was competitive for the artificial ubiquinone.

Compound 1:

1H NMR (CDCl3), 8.35 (1H, dd, J = 1.23 and 8.11), 7.62 (1H, m), 7.40 (1H, d, 8.17), 7.30 (1H, m), 6.12 (1H, s), 2.51 (2H, s), 1.80 (4H, bd, J =10.38), 1.70 (2H,bd, J=11.38), 1.00-1.30 (5H, m), 0.94 (6H, s). [M+H]+ 284 (100%) 284.2012 $C_{19}H_{26}NO$ calc. 284.2014, [M+Na]+306 (90%) 306.1835 C19H25NONa calc. 306.1834.

Compound 2:

^{1}H NMR (400MHz, $CDCl_3$) δ_H 10.45 (t, 1H, J = 5.8 Hz, NH), 8.75 (s, 1H), 8.01 (d, 1H, J =13.1 Hz), 7.54 (s, 1H), 7.36-7.26 (m, 4H), 7.09 (d, 1H, J = 3.5 Hz), 6.82 (d, 1H, J = 6.9 Hz), 6.52 (dd, 1H, J = 3.5 Hz, 1.8 Hz), 4.62 (d, 2H, J = 5.9 Hz, CH_2Ar), 4.28 (q, 2H, J = 7.2 Hz, NCH_2), 4.06 (bs , 4H, NCH_2), 3.35 (t, 4H, J = 4.8 Hz, CH_2N), 1.54 (t, 3H, J = 7.2 Hz, CH_3) ^{13}C NMR (100MHz, $CDCl_3$), δ_C 175.7, 165.5, 159.5, 154.8, 152.4, 148.1, 147.2, 145.1, 144.4, 137.9, `36.8, 133.2, 129.4, 129.1, 123.4, 117.6, 113.6, 112.0, 104.5, 50.7, 49.6, 42.9, 14.9 MS (ES+), [M + Na] $^{+}$ (100), 559.0 HRMS calculated for 559.1524 $C_{28}H_{26}N_4O_4FClNa$, found 559.1517.

Compound 3: ^{1}H NMR (300MHz, DMSO-d6) δ_H 12.52 (br s, 1H, NH), 10.85 (br s, 1H, NH), 8.35 (s, 1H, =CH), 7.11-7.60 (m, 9H, ArH), 4.55 (q, 1H, J = 7.1 Hz, NCH), 1.32-1.95 (m, 10H) MS (ES+), [M + Na] + (100), 559.0 HRMS calculated for $[M + H]^+$ 414.1812 $C_{25}H_{24}N_3O_3$, found 559.1812.

Compound 4:

^{1}H NMR (400MHz, $CDCl_3$) δ_H 8.42 (d, 1H, J = 7.8 Hz, Ar), 8.20 (d, 1H, J = 8.4 Hz, Ar), 7.91 (s, 1H, Ar), 7.81 (t, 1H, J = 7.0 Hz, Ar), 7.74 (t, 1H, J = 8.1 Hz, Ar), 7.54 (d, 1H, J = 8.3 Hz, Ar), 7.52 (s, 1H, Ar), 6.58 (d, 1H, J = 8.2 Hz, Ar), 3.68 (s, 3H, NCH_3), 2.77 (s, 3H, SCH_3) ^{13}C NMR (100MHz, $CDCl_3$), δ_C 155.8, 153.5, 145.4, 142.7, 135.3, 131.1, 128.8, 127.5, 126.5, 126.1, 124.2, 122.6, 119.6, 111.5, 109.4, 30.6, 14.5 MS (ES+), [M + H] $^{+}$ (100), 389.0, HRMS calculated for 389.0282 $C_{19}H_{15}N_2OSCl_2$, found 389.0264.

Compound 5: ^{1}H NMR (300MHz, DMSO-d6) δ_H 10.05 (s, 1H, NH), 8.39 (s, 1H, Ar), 8.19 (d, 1H, J = 8.4 Hz, Ar), 7.75-7.85 (m, 2H, Ar), 7.70 (d, 1H, J = 8.4 Hz, Ar), 7.55 (m, 1H, J = 7.2 Hz, Ar), 7.20-7.40 (m, 2H, Ar), 3.87 (d, 2H, NCH_2), 2.20-2.40 (m, 1H), 1.75-1.95 (m, 4H), 1.65-1.75 (m, 1H), 1.30-1.45 (m 2H), 1.20-1.01 (m, 2H), HRMS calculated for 398.1657 $C_{11}H_{24}F_4N_5O_6$, found 389.1665.

The ranks of the hit compounds presented are given in the table below. Compounds 1 and 2 came from the initial screening (substructure search) and so do not have rankings. There are two rankings presented – one

from the set of compounds before diversity selection procedures (32k set) and one from after the diversity selection procedure (16k set).

Molecule	Rank in 32k set	Rank in 16k set
1	NA	NA
2	NA	NA
3	19163	10452
4	18273	10892
5	8700	5543

NA – Not Applicable