SUPPLEMENTAL METHODS, TABLES AND FIGURES

Tetrahydro-2-naphthyl and 2-indanyl triazolopyrimidines targeting *Plasmodium falciparum* dihydroorotate dehydrogenase display potent and selective antimalarial activity

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Contents

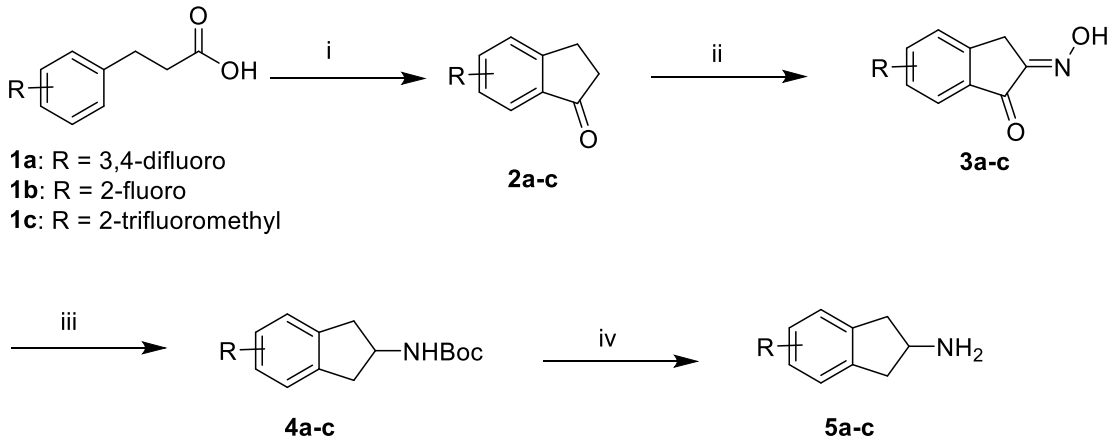
Page Number: Title of Table or Figure

SUPPLEMENTARY METHODS

CHEMISTRY

5a-c inden-2-amine precursors were synthesized by the reported procedure "U.S. Pat. Appl. 20100113512, 06, May 2010"

Scheme S1: Synthesis of substituted inden-2-amine precursors 5a-c.[a]



[a]Reagents and conditions: (i) $ClSO_3H$, RT, 1h; (ii) acetyl chloride, isoamyl nitrite, MeOH, DCM, Heptane (iii) (a) Pd/C,50 psi, AcOH, H_2SO_4, 60 ^oC, 12h; (b) Boc,TEA,DCM; (iv) HCl, Dioxane, RT, 2h

5,6-difluoro-2,3-dihydro-1H-inden-1-one (2a). 3-(3,4-difluorophenyl)propanoic acid (**1a**) (5.0 g, 26 mmol) was dissolved in chlorosulfonic acid (47.0 g, 402 mmol) at 0 ^oC. The reaction mixture was stirred at 25 °C for 1 h. After the reaction was complete, the reaction mixture was quenched by the slow addition of water at 0 ^oC, and then DCM (50 mL) was added. The two layers were separated, the aqueous layer extracted with DCM and the organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain 4.1 g (91% yield) of 5,6-difluoro-2,3-dihydro-1H-inden-1-one (**2a**) as a pale yellow solid.

^1H NMR (400 MHz, CDCl3): δ 7.53 (t, J = 8.24 Hz, 1H), 7.26 (t, J = 7.84 Hz, 1H), 3.14 – 3.11 (m, 2H), 2.75 – 2.72 (m, 2H).

(E)-5,6-difluoro-2-(hydroxyimino)-2,3-dihydro-1H-inden-1-one (3a): Acetyl chloride (3.4 mL) was added drop wise to the mixture of methanol (2.2 mL), DCM (29 mL), heptane (19 mL) at 0 ^{o}C. After 15min, 5,6-difluoro-2,3-dihydro-1H-inden-1-one (**2a**) (4.1 g, 16 mmol) in DCM(12 mL) was added drop wise, followed by 3-methyl butyl nitrite (3.7 g, 22 mmol). The reaction mixture was stirred at room temperature for 2 h. Solid precipitated was collected by filtration and dried under vacuum to obtain 3.8 g (79% yield) of (E)-5,6-difluoro-2-(hydroxyimino)-2,3-dihydro-1H-inden-1-one (**3a**) as off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.76 (s, 1H), 7.84 –7.72 (m, 2H), 3.75 (s, 2H). m/z =196.2 (M-1)

Tert-butyl (5,6-difluoro-2,3-dihydro-1H-inden-2-yl)carbamate (4a)

To a solution of (E)-5,6-difluoro-2-(hydroxyimino)-2,3-dihydro-1H-inden-1-one (**3a**) (2.0 g, 10 mmol) in acetic acid (60 mL), H_2SO_4 (4 mL) was added palladium on carbon(1.2 g, 11 mmol). The reaction mixture was hydrogenated at 50 psi at 60 ^{o}C for 12 h. After the completion reaction the reaction mixture was cooled, filtered over celite and washed with methanol. The filtrate concentrated under reduced pressure. Residue was dissolved in DCM (5mL), neutralized with triethylamine (2.8 g) and BOC anhydride (3 g) was added and stirred for 1h at 25 °C. Reaction mixture was washed with water and brine solution and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography to obtain 0.37 g (13.7% yield) of tert-butyl (5,6-difluoro-2,3-dihydro-1H-inden-2-yl)carbamate (**4a**).

5,6-difluoro-2,3-dihydro-1H-inden-2-amine hydrochloride (5a)

Tert-butyl (5,6-difluoro-2,3-dihydro-1H-inden-2-yl)carbamate was dissolved in DCM (4 mL) HCl-Dioxane (10 mL) was added drop wise at 0 ^{o}C and stirred at 25 °C for 2 h. The volatiles

were removed under reduced pressure to obtain 0.25 g (90% yield) of 5,6-difluoro-2,3-dihydro-1H-inden-2-amine hydrochloride. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.11 (s, 2H), 7.38 – 7.34 (m, 2H), 4.05 – 4.01 (m, 1H) 3.28 – 3.22 (m, 2H), 2.92 – 2.87 (m, 2H); m/z =170 $(MH)^+$.

4-fluoro-2,3-dihydro-1H-inden-2-amine hydrochloride (5b).

The compound was prepared following similar procedures to the ones followed for **5a**, except for the synthesis of intermediate **2b**, which was:

4-fluoro-2,3-dihydro-1H-inden-1-one (2b): To a solution of 3-(2-fluorophenyl)propanoic acid (**1b**) (5.0 g, 29 mmol) in DCM at 0 ^oC was added oxalyl chloride (5.4 g, 44 mmol) followed by 2-3 drops of DMF. The resulting mixture was stirred until no more gas evolution was observed. After concentration of the reaction mixture, the residue was dissolved in DCM (35 mL), cooled to 0 ^oC and $AlCl_3$ (3.8 g, 29 mmol) was added in 3 portions at 3 minute intervals. After stirring for 1 h, the reaction mixture was quenched in ice water and the layers were separated. The aqueous layer was extracted twice with ether and the combined organic extracts were washed with water, saturated $NaHCO_3$, brine solution, dried over Na_2SO_4 and concentrated under reduced pressure to obtain 4.1 g (91.9% yield) of 4-fluoro-2,3-dihydro-1H-inden-1-one (**2b**).

4-fluoro-2,3-dihydro-1H-inden-2-amine hydrochloride (5b): ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.30 (s, 2H), 7.29 (d, J = 8.32 Hz, 1H), 7.14 (d, J = 9.12 Hz, 1H), 7.04 – 6.99 (m, 1H), 4.04 – 4.00 (m, 1H), 3.30 – 3.20 (m, 2H), 3.01 – 2.90 (m, 2H); m/z =152 $(MH)^+$.

4-(trifluoromethyl)-2,3-dihydro-1H-inden-2-amine hydrochloride (5c). The compound was prepared following similar procedures to the ones followed for **5a**.

4-(trifluoromethyl)-2,3-dihydro-1H-inden-2-amine hydrochloride (5c): ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.37 (s, 2H), 7.61 –7.54 (m, 2H), 7.44 (d, J = 7.64 Hz, 1H), 4.10 – 4.06 (m, 1H), 3.46 – 3.37 (m, 2H), 3.17 – 3.03 (m, 2H); m/z =202.2 $(MH)^+$.

SUPPLEMENTAL TABLES

Table S1: Crystal data and structure refinement for **9** (Enantiomer-I).

Empirical formula	C18 H18 Cl F2 N5	
Formula weight	377.82	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 2_1	
Unit cell dimensions	a = 8.4266(16) Å	α= 90°.
	b = 29.117(5) Å	β= 90.876(10)°.
	c = 13.891(3) Å	γ = 90°.
Volume	3407.8(11) $Å^3$	
Z	8	
Density (calculated)	1.473 Mg/m^3	
Absorption coefficient	0.258 mm^{-1}	
F(000)	1568	
Crystal size	0.20 x 0.13 x 0.10 mm^3	
Theta range for data collection	1.62 to 28.33°.	
Index ranges	-11<=h<=11, -38<=k<=38, -18<=l<=18	
Reflections collected	113483	
Independent reflections	16915 [R(int) = 0.0703]	
Completeness to theta = 25.00°	99.9 %	
Max. and min. transmission	0.9747 and 0.9503	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	16915 / 1 / 945	
Goodness-of-fit on F^2	1.021	
Final R indices [I>2sigma(I)]	R1 = 0.0409, wR2 = 0.0822	
R indices (all data)	R1 = 0.0587, wR2 = 0.0895	
Absolute structure parameter	-0.01(3)	
Largest diff. peak and hole	0.357 and -0.297 $e.Å^{-3}$	

Table S2. Activity of select compounds on *P. falciparum* drug resistant strains

Cmpd	EC_{50}, nM					
	3D7	**Dd2**	**HB3**	**D6**	**K1**	**TM90C2B**
35	40	43	21	55	49	36
9	1.2	1.2	0.47	1.5	1.7	1.3

The tested strains have resistance to the following agents:

3D7 - sulfadoxine

Dd2 – chloroquine, quinine, mefloquine, pyrimethamine, cycloguanil, sulfadoxine

HB3 – pyrimethamine

D6 - none

K1 – chloroquine, pyrimethamine, cycloguanil

TM90C2B - atovaquone, chloroquine, cycloguanil, pyrimethamine

Table S3. *Pf*DHODH-**13** X-ray diffraction data and refinement statistics.

Data Collection	
Resolution (Å)	31.6 - 2.32 (2.36 - 2.32)
Space group	$P6_4$
Cell dimensions (Å)	*a*=*b*=86.1, *c*= 139.1
Wavelength (Å)	0.97918
Unique reflections	25,176 (1,224)
Data Completeness (%)	99.5 (99.3)
Redundancy	12.5 (10.9)
I/σ	43.4 (1.0)
R_{merge} (%)[a]	4.8 (100)
R_{pim} (%)[b]	1.7 (68.9)
Wilson B-value (Å^2)	30.7
Refinement	
Resolution (Å)	31.6 - 2.32 (2.43 - 2.32)
Unique reflections	21,301 (1021)
Data Completeness (%)	84.4 (32.5)
R_{work} / R_{free} (%)	17.7 (22.5)/ 21.1(25.2)
No. of atoms/ water	2966 /65
rms deviations	
Bond length (Å)	0.004
Bond angles (deg.)	0.85
Mean B (Å^2)	49.3
Ramachandran plot[c]	
Favored/Allowed (%)	96.1/3.9
Missing residues	159-160, 344-354, 567-569

Data for the outermost shell are given in parentheses.

[a] $R_{merge} = 100\ \Sigma_h\Sigma_i|I_{h,i} - \langle I_h\rangle|/\Sigma_h\Sigma_i\ \langle I_{h,i}\rangle$, where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.

[b] $R_{pim} = 100\ \Sigma_h\Sigma_i\ [1/(n_h - 1)]^{1/2}|I_{h,i} - \langle I_h\rangle|/\Sigma_h\Sigma_i\langle I_{h,i}\rangle$, where n_h is the number of observations of reflections **h**[1]

[c] As defined by the validation suite MolProbity[2]

1. Evans, P. R., An introduction to data reduction: space-group determination, scaling and intensity statistics. *Acta Crystallogr D Biol Crystallogr* **2011,** *67* (Pt 4), 282-92.
2. Chen, V. B.; Arendall, W. B., 3rd; Headd, J. J.; Keedy, D. A.; Immormino, R. M.; Kapral, G. J.; Murray, L. W.; Richardson, J. S.; Richardson, D. C., MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D Biol Crystallogr* **2010,** *66* (Pt 1), 12-21.

Table S4. SCID mouse blood pharmacokinetics

Cmpd	**Target Dose (mg/Kg)**	**C_{max} (µM)**	**T_{max} (h)**	**$AUC_{(0\text{-}last)}$ µM.h**
35	10 (14)	10.3	8	124[a]
	30 (35.2)	14.5	2	137[a]
9	10	0.77	4	4.1[b]
	30 (36.8)	2.83	4	28.0[a]
7	10	1.19	4	6.51[b]
	30	2.20	2	22.8[a]

[a]t=23 h, [b]t= 8 h. Doses in parenthesis are measured doses where different from the target dose.

SUPPLEMENTAL FIGURES

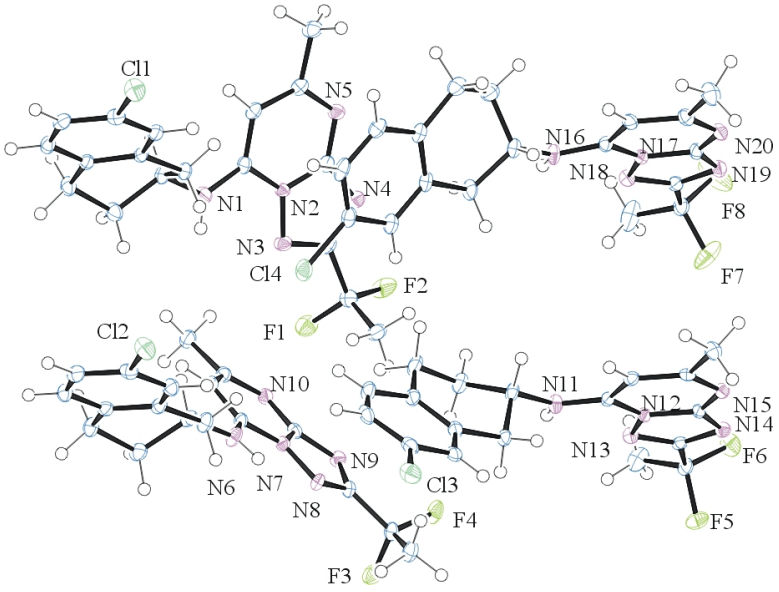


Fig. S1. ORTEP of **9** Enantiomer-I structure with thermal ellipsoids at the 50% probability level.

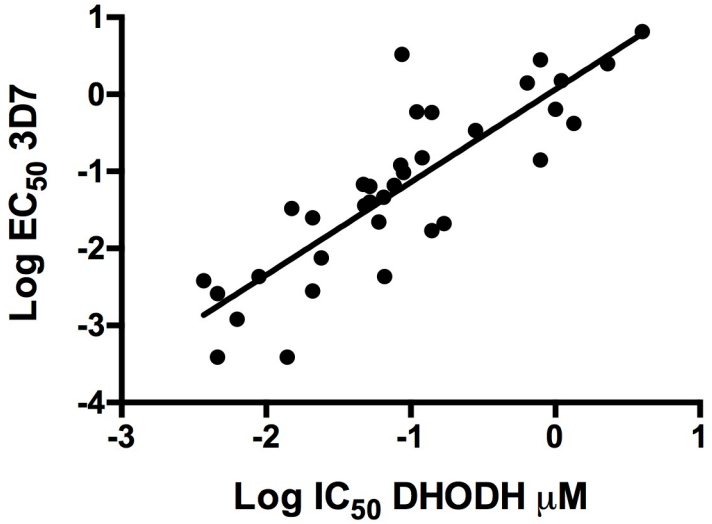


Fig. S2. Correlation of *Pf*DHODH acivity versus *P. falciparum* 3D7 activity. The log of the EC_{50} obtained on *P. falciparum* 3D7 cells is plotted versus the log of the IC_{50} for *Pf*DHODH. Data are taken from Table 1. The slope of the correlation line is 1.2 ± 0.13 and the R square is 0.73.

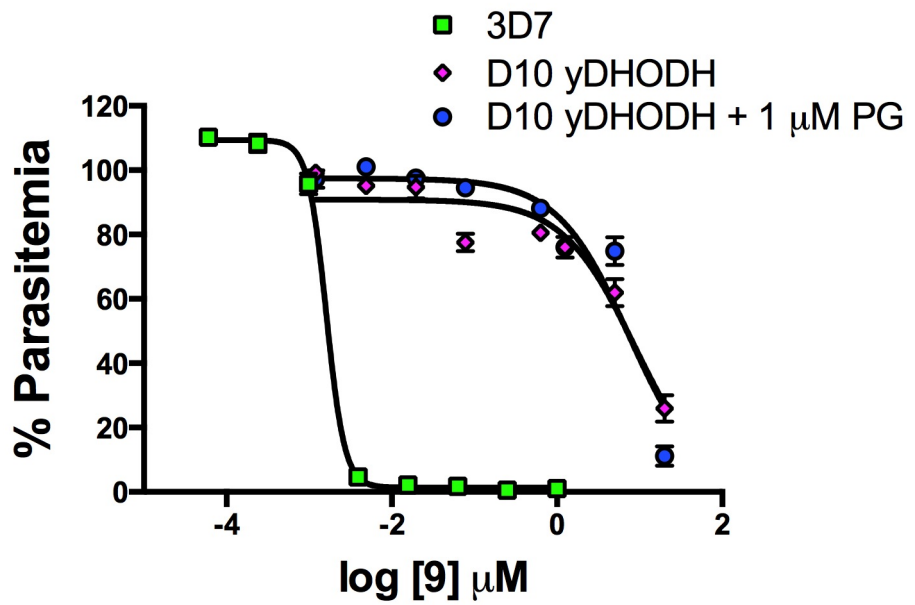


Fig. S3. *In vitro P. falciparum* growth inhibition comparing activity on wild-type 3D7 and D10 yDHODH parasites. The dose response curves for the D10 yDHODH line were done ± 1 μM proguanil. 3D7 data were fitted to log (I) vs response – variable slope 4 parameter fit in Graph Pad Prism and D10 yDHODH data were fitted toY= Yo/(1+(X/IC50)), where X is the concentration of inhibitor. Error bars represent the standard deviation of the mean for 3 replicates. The fitted EC_{50}'s: 95% confidence interval were: 3D7 (0.0016 μM; 95% CI 0.0011 – 0/0013); D10 yDHODH (8.6 μM; 95% CI 6.0 – 11.3); D10 yDHODH + proguanil (PG) (7.4 μM; 95% CI 4.7 – 10)

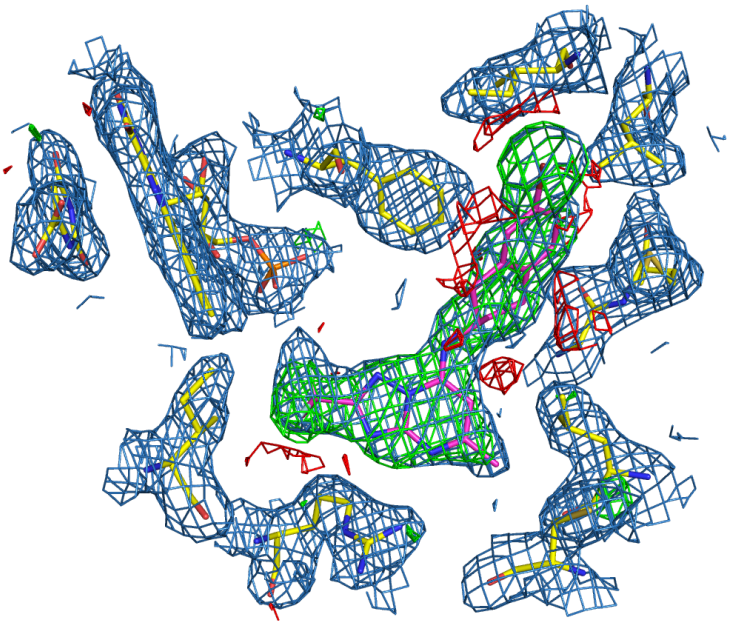


Fig. S4A. Electron density map of *Pf*DHODH-**13** binding site. $2F_o - F_c$ electron density map (blue) of the fully refined structure contoured at 1.0 σ. Fo-Fc map (green), contoured at 3 σ showing the positive density for the compound prior to refinement. Negative density Fo-Fc map (red) contoured at -3 σ.

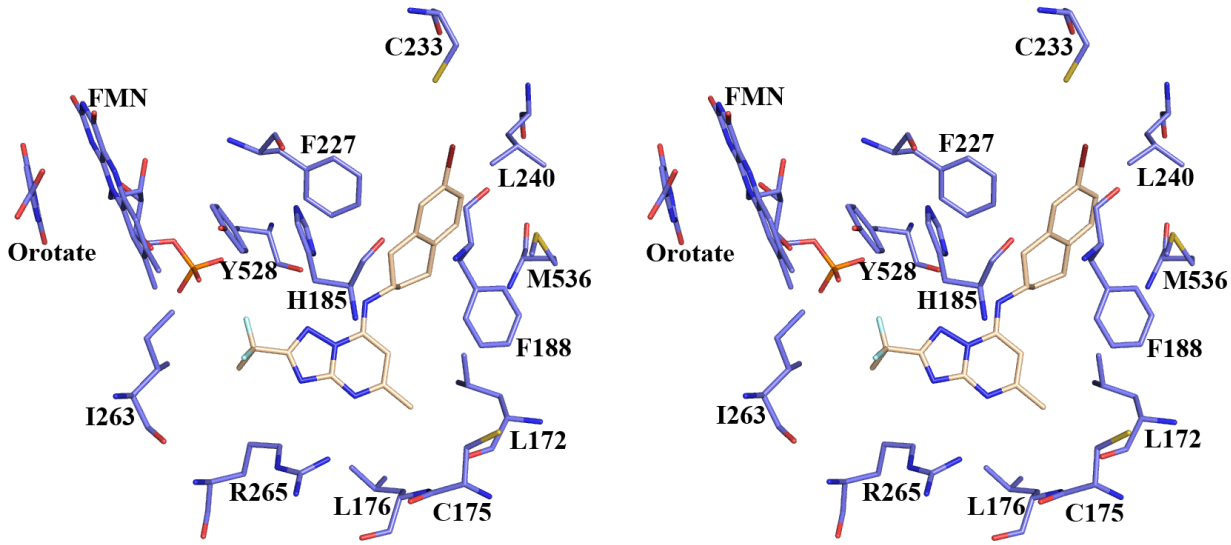


Fig. S4B. Stereo diagram of the *Pf*DHODH-**13** x-ray structure showing the inhibitor binding-site. Select residues within the 4Å inhibitor shell are shown. **13** is shown in tan, protein carbons are shown in purple, nitrogens are blue, oxygens are red, sulfur is light yellow, fluorines are light blue, and bromine is deep red.