Supporting information

Evaluation of aminohydantoins as a novel class of antimalarial agents

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Table S1. Profile of Lead Compounds

Compound	8p	9a	9f	9g
MW	393	395	408	409
cLogP	4.0	2.1	4.3	2.6
Pf 3D7 IC_{50} (μM, 72h)	0.463	0.459	0.383	0.463
Pf Dd2 IC_{50} (μM, 72h)	0.480	0.526	0.367	0.442
Pf 3D7 IC_{50} (μM, 48h)	0.751	0.404	0.339	0.571
HepG2 IC_{50} (μM)	9.4	>50	8.0	30
CYP1A2 IC_{50} (μM)	>10	nd	nd	nd
CYP2C19 IC_{50} (μM)	5.48	nd	nd	nd
CYP2D6 IC_{50} (μM)	>10	nd	nd	nd
CYP3A4 IC_{50} (μM)	0.449	nd	nd	nd
MLM $t_{1/2}$ (min)	81	nd	26	nd
RLM $t_{1/2}$ (min)	61	83	14	35
HLM $t_{1/2}$ (min)	29	36	nd	nd
hPPB (%)	98.5	78.1	99.7	88.7
Rat PK $t_{1/2}$ (h)	2.9	1.1	1.2	nd
Rat PK oral bioavailability (%F)	16	21	nd	nd

nd = not determined.

Table S2. Protease selectivity profiles

Cmpd	IC_{50} (μM)					
	Pf 3D7	PM-II	PM-IV	BACE	CatD	CatE
4	2.76	0.012	0.045	2.3	0.385	0.099
7a	>10	>10	>10	nd	nd	nd
7b	>5	>10	nd	nd	nd	nd
8p	0.463	0.004	0.015	12.0	>10	1.19
9a	0.453	0.007	0.018	~10	>10	0.511
9b	14.8	0.594	0.243	nd	nd	nd
9c	>5.0	0.505	0.192	nd	nd	nd
9d	0.697	0.035	0.109	nd	nd	nd
9f	0.383	0.002	0.010	6.01	0.52	0.233
9g	0.463	0.017	0.059	2.07	0.934	0.841
10a	0.768	0.036	0.041	28.9	1.10	1.86
(±)-10k	0.595	0.007	0.007	~7.15	~14.9	4.01
(±)-10m	0.75	0.013	0.031	18.1	4.38	2.44
(±)-11	6.88	1.01	0.323	1.68	>10	>10

nd = not determined.

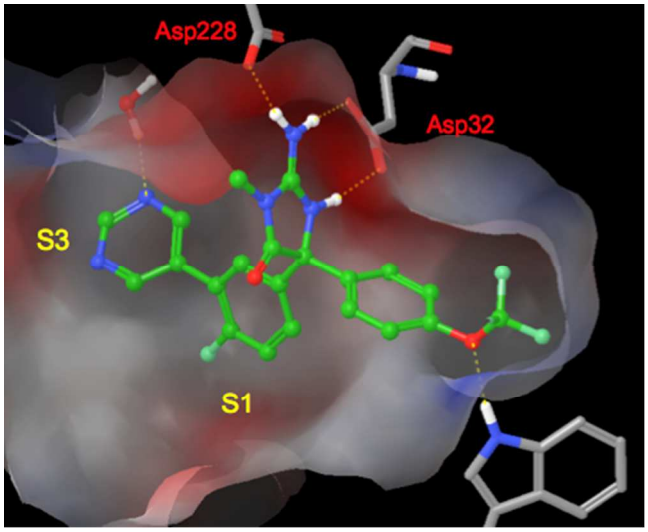


Figure S1. Aspartic protease BACE binding site of aminohydantoins adapted from 3INH.[1]

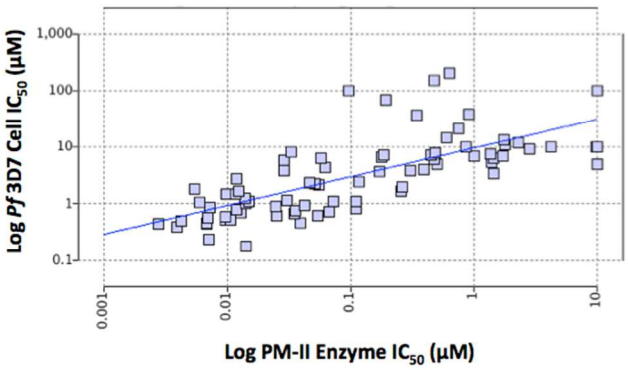
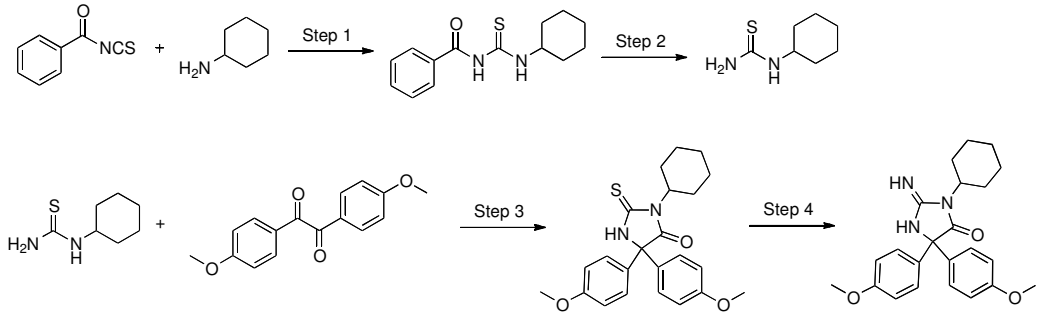


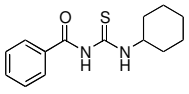
Figure S2. General correlation between PM-II enzyme activity and antimalarial 3D7 activity.

General Synthesis Methods. All materials were obtained from commercial sources and used as purchased. Chromatography solvents were chromatography grade and were used without further purification. Thin layer chromatography (TLC) analysis was performed using Merck silica gel 60 F-254 thin layer plates. LC-MS analyses were performed on an Agilent 1200HPLC/MCD electrospray mass spectrometer in positive ion mode. The scan range was 100-1000d. Preparative reverse phase HPLC was performed on a SHIMADZU LC-20AP equipped with a C18 column and a methanol/water gradient. The purity of tested compounds was ≥95% as determined by HPLC analysis conducted on an Agilent 1260 system using a reverse phase C18 column with diode array detector unless stated otherwise. NMR spectra were recorded on a Bruker 400 MHz spectrometer. The signal of the deuterated solvent was used as internal reference. Chemical shifts (δ) are given in ppm and are referenced to residual not fully deuterated solvent signal. Coupling constants (J) are given in Hz.

Synthesis of 3-cyclohexyl-2-imino-5,5-bis(4-methoxyphenyl)imidazolidin-4-one (8p)

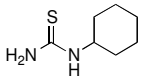


Step 1. Preparation of *N*-(cyclohexylcarbamothioyl)benzamide



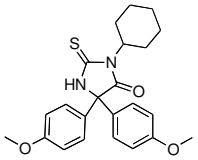
Benzoyl isothiocyanate (19.4 g, 118.9 mmol) was dissolved in dichloromethane and cooled to 0 °C. Cyclohexylamine (13 g, 131.1 mmol) was added dropwise. Then the reaction was stirred at room temperature for 2h. The mixture was washed with water, dried over sodium sulphate. Concentration under vaccum gave the title compound as a pale white solid in quantitative yield.

Step 2. Preparation of 1-cyclohexylthiourea



To a solution of *N*-(cyclohexylcarbamothioyl)benzamide (17.1 g, 65.5 mmol) in ethanol was added aqueous potassium carbonate (18.2 g, 132.2 mmol). The mixture was stirred at reflux temperature for 4h. The organic layer was concentrated to a small volume, after which the product could be collected by filtration, then washed with petroleum ether and dried in vacuo to give the title compound as a white solid (9.8 g, 95% two steps).

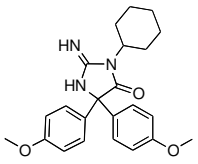
Step 3. Preparation of 3-cyclohexyl-5,5-bis(4-methoxyphenyl)-2-thioxoimidazolidin-4-one (7b)



1-Cyclohexylthiourea (1.3 g, 8.2 mmol) and 4,4′-dimethoxybenzil (CAS 1226-42-2; 2 g, 7.4 mmol) were dissolved in dimethyl sulfoxide and the solution was heated to 110 °C. An aqueous solution of potassium hydroxide (0.62 g, 11.1 mmol) was added drop wise and the resulting mixture was stirred for 10 min, allowed to cool, and then extracted with dichloromethane. The combined organic phases were washed with water, dried over sodium sulphate and concentrated in vacuo to give the title compound as a residue which was used without further purification for Step 4.

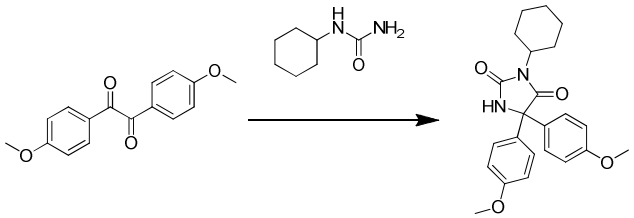
A sample was prepared for biological analysis as follows: 1-Cyclohexylthiourea (0.28 g, 1.77 mmol) and 4,4′-dimethoxybenzil (CAS 1226-42-2; 0.4 g, 1.48 mmol) were dissolved in dimethyl sulfoxide and the solution was heated to 110 °C. An aqueous solution of potassium hydroxide (0.13 g, 2.2 mmol) was added drop wise and the resulting mixture was stirred for 10 min, allowed to cool, and then extracted with dichloromethane. The combined organic phases were washed with water, dried over sodium sulphate and concentrated in vacuo. The residue was purified over a silica column to give the title compound as a white solid (0.56g, 97%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.49 (s, 1H), 7.16 (d, J=8.8Hz, 4H), 6.97 (d, J=8.8 Hz, 4H), 4.50 (m, 1H), 3.75 (s, 6H), 2.11 (m, 2H), 1.78 (m, 2H), 1.59 (m, 3H), 1.05-1.31 (m, 3H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 181.2, 174.1, 159.1, 130.4, 127.9, 114.0, 69.3, 55.2, 53.9, 28.1, 25.3, 24.7. LC-MS m/z 395.2 $(M+H)^+$. HPLC t_R = 13.04 min, purity 99.4%. HRMS calcd for $C_{23}H_{27}N_2O_3S$ 411.1737; found 411.1765.

Step 4. Preparation of 3-cyclohexyl-2-imino-5,5-bis(4-methoxyphenyl)imidazolidin-4-one (8p)



To a solution of 3-cyclohexyl-5,5-bis(4-methoxyphenyl)-2-thioxoimidazolidin-4-one (3.0 g, 7.3 mmol) in methanol was added aqueous ammonium (25%, 40 ml) and tert-butyl hydroperoxide (70%, 10 ml) separately, and the resulting solution was stirred at room temperature overnight. The mixture was extracted with dichloromethane and the organic layer was concentrated in vacuo. The residue was purified over a silica column to give the title compound as a white solid (1.7g, 59% two steps). ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.25 (d, *J*=8.0 Hz, 4H), 6.84 (d, *J*=7.6 Hz, 4H), 6.47 (s, 1H), 3.71 (s, 6H), 3.70 (s, 1H), 2.09 (m, 2H), 1.74 (m, 2H), 1.52-1.60 (m, 3H), 1.26 (m, 2H), 1.13 (m, 1H). LC-MS m/z 411.1 $(M+H)^+$. ^{13}C NMR (125 MHz, d_6-DMSO) δ 181.1, 158.0, 154.9, 135.3, 127.8, 113.2, 73.45, 55.0, 51.9, 28.8, 25.3, 24.6. HPLC t_R = 7.06 min, purity 98.8%. HRMS calcd for $C_{23}H_{28}N_3O_3$ 394.2125; found 394.2125.

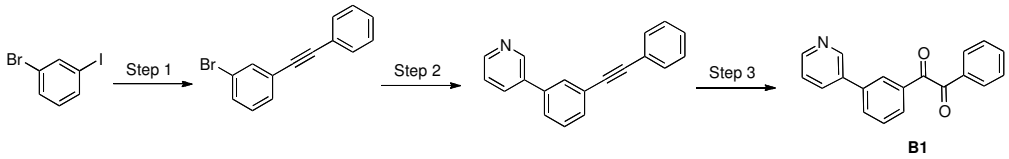
Synthesis of 3-cyclohexyl-5,5-bis(4-methoxyphenyl)imidazolidine-2,4-dione (7a)



1-Cyclohexylurea (CAS 698-90-8; 0.25 g, 1.76mmol) and 4,4′-dimethoxybenzil (CAS 1226-42-2; 0.4 g, 1.48 mmol) were dissolved in dimethyl sulfoxide and the solution was heated to 110 °C. An aqueous solution of potassium hydroxide (0.13 g, 2.2 mmol) was added drop wise and the resulting mixture was stirred for 10 min, allowed to cool, and then extracted with dichloromethane. The combined organic phases were washed with water, dried over sodium sulphate and concentrated in vacuo. The residue was purified over a silica column to give the title compound as a white solid (0.58g, 98%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.40 (s, 1H), 7.20 (d, J=8.8Hz, 4H), 6.94 (d, J=8.8 Hz, 4H), 3.80 (m, 1H), 3.74 (s, 6H), 2.04 (m, 2H), 1.55 (m,

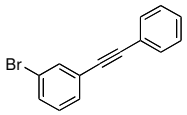
2H), 1.59 (m, 3H), 1.06-1.30 (m, 3H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 173.6, 158.9, 155.2, 132.0, 127.8, 113.8, 67.3, 55.1, 50.5, 29.0, 25.2, 24.8. LC-MS m/z 395.2 $(M+H)^+$. HPLC t_R = 8.70 min, purity 99.1%. HRMS calcd for $C_{23}H_{27}N_2O_4$ 395.1965; found 395.2010.

Representative Synthesis of Non-symmetrical Diketones. Preparation of 1-phenyl-2-(3-(pyridin-3-yl)phenyl)ethane-1,2-dione (B1)



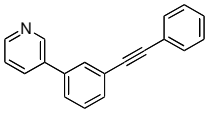
B1

Step 1. Preparation of 1-bromo-3-(phenylethynyl)benzene



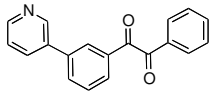
1-bromo-3-iodobenzene (15.3 g, 54.0 mmol), phenylacetylene (5 g, 49.0 mmol), tetrakis(triphenylphosphine)palladium (2.8 g, 2.4 mmol) and copper(I) iodide (0.46g, 2.4 mmol) were dissolved in dry toluene and diisopropylamine (35 ml, 196 mmol) under a nitrogen atmosphere. The reaction was stirred at room temperature overnight. The mixture was extracted with ethyl acetate and the organic layer was dried in vacuo to give the title compound as a residue that was used without further purification.

Step 2. Preparation of 3-(3-(phenylethynyl)phenyl)pyridine



1-Bromo-3-(phenylethynyl)benzene (3 g, 11.7 mmol), 3-pyridylboronic acid(1.6 g, 13.0 mmol), Tetrakis(triphenylphosphine)palladium(0.3 g, 0.26 mmol) and potassium carbonate (3.2 g, 23.2 mmol) were dissolved in N,N-dimethylformamide and the reaction was stirred at 100 °C overnight under nitrogen. The mixture was extracted with dichloromethane and the combined organic phases were washed with water, dried over sodium sulphate and concentrated in vacuo to give the title compound as oil (1.3 g, 43% yield).

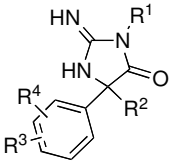
Step 3. Preparation of 1-phenyl-2-(3-(pyridin-3-yl)phenyl)ethane-1,2-dione



B1

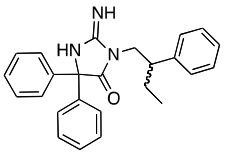
To a solution of 3-(3-(phenylethynyl)phenyl)pyridine (5.4 g, 21.2 mmol) in dimethyl sulfoxide was added palladium chloride (0.87 g, 4.9 mmol) under nitrogen. The reaction was heated to 110 °C overnight. The mixture was extracted with dichloromethane. The combined organic phases were washed with water, dried over sodium sulphate and concentrated in vacuo. The residue was purified over a silica column to give the title compound as a white solid (2.3 g, 38% yield).

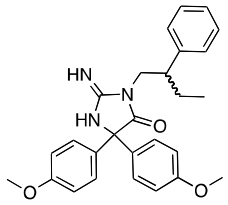
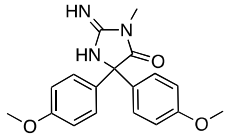
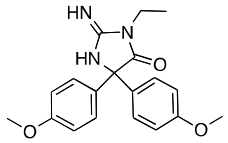
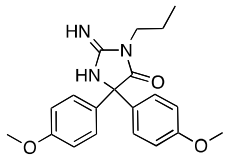
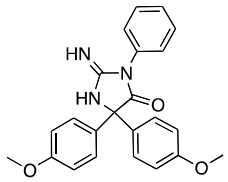
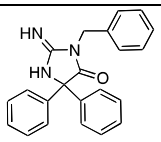
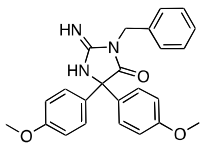
Preparation of Aminohydantoin Analogs

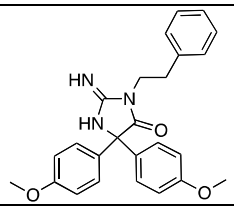
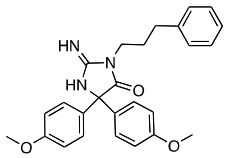
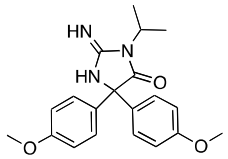
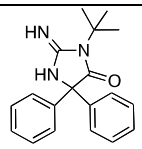
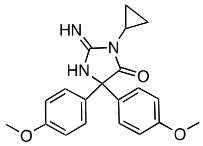
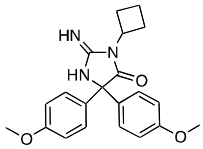
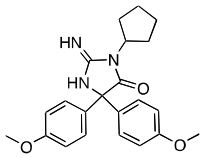


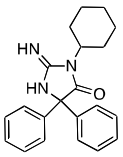
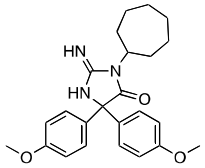
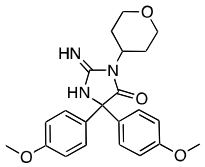
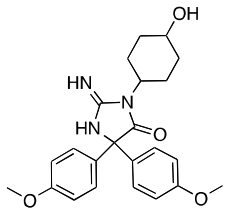
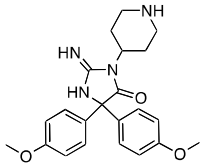
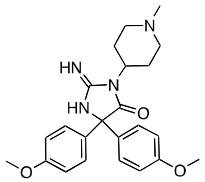
Aminohydantoins (Table S1) were prepared according to the procedure for compound **8p** from the corresponding thiourea intermediate and corresponding diketone.

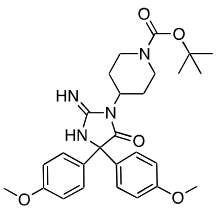
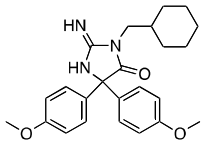
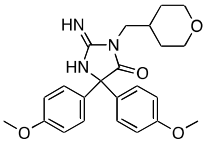
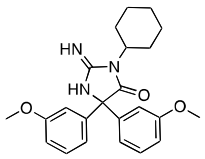
Table S1. Examples 4,8-11.

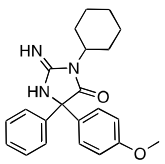
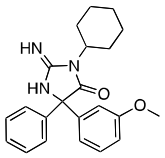
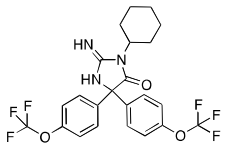
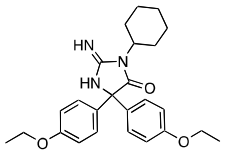
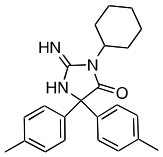
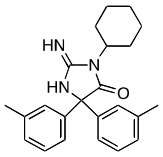
Ex.	IUPAC Name	Structure	Yield (%)	^{1}H NMR (400 MHz, DMSO-d_6) δ	LC-MS m/z $(M+H)^+$	HPLC t_R (min) / Purity (%)
4	2-imino-5,5-diphenyl-3-(2-phenylbutyl)imidazolidin-4-one		35	7.13-7.24 (m, 13H), 7.01 (s, 2H), 6.56 (s, 1H), 3.60-3.73 (m, 2H), 2.93 (s, 1H), 1.46-1.62 (m, 2H), 0.63 (t, *J*=6.8, 7.6Hz, 3H)	384.2	8.41 / 96

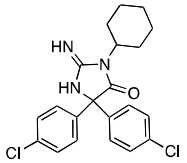
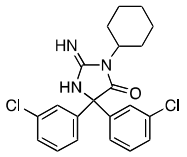
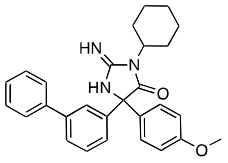
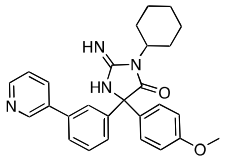
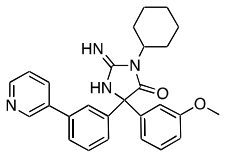
Ex.	IUPAC Name	Structure	Yield (%)	^{1}H NMR (400 MHz, DMSO-d_6) δ	LC-MS m/z $(M+H)^+$	HPLC t_R (min) / Purity (%)
8a	2-imino-5,5-bis(4-methoxyphenyl)-3-(2-phenylbutyl)imidazolidin-4-one		35	δ 7.14-7.22 (m, 7H), 6.90 (d, *J*=8.4 Hz, 2H), 6.82 (d, *J*=8.8 Hz, 2H), 6.73 (d, *J*=8.8Hz, 2H), 6.47 (s, 1H), 3.70 (d, *J*=2.8 Hz, 6H), 3.63 (m, 2H), 2.94 (s, 1H), 1.44-1.64 (m, 2H), 0.63 (t, *J*=7.2Hz, 3H)	444.2	7.79 / 97
8b	2-imino-5,5-bis(4-methoxyphenyl)-3-methylimidazolidin-4-one		50	7.30 (d, *J*=8.4Hz, 4H), 6.83 (d, *J*=8.4 Hz, 4H), 6.55 (s, 1H), 3.70 (s, 6H), 2.96 (s, 3H)	326.1	4.27 / 99
8c	3-ethyl-2-imino-5,5-bis(4-methoxyphenyl)imidazolidin-4-one		55	7.27 (d, *J*=8.8Hz, 4H), 6.84 (d, *J*=8.4 Hz, 4H), 6.57 (s, 1H), 3.70 (s, 6H), 3.50 (q, *J*=6.8, 14 Hz, 2H), 1.04 (t, *J*=6.8, 7.2Hz, 3H)	340.1	4.50 / 95
8d	2-imino-5,5-bis(4-methoxyphenyl)-3-propylimidazolidin-4-one		55	δ 7.28 (d, *J*=8.8Hz, 4H), 6.84 (d, *J*=8.8 Hz, 4H), 6.55 (s, 1H), 3.71 (s, 6H), 3.41 (t, *J*=6.8, 7.2 Hz), 1. 47 (m, 2H), 0.77 (t, *J*=7.2, 7.6 Hz, 3H)	354.1	4.92 / 96
8e	2-imino-5,5-bis(4-methoxyphenyl)-3-phenylimidazolidin-4-one		45	7.43-7.53 (m, 3H), 7.36 (dd, *J*=2, 7.2 Hz, 4H), 7.25 (d, *J*=6.8 Hz, 2H), 6.89 (d, *J*=8.4 Hz, 4H), 6.34 (s, 1H), 3.73 (s, 6H)	388.1	4.79 / 97
8f	3-benzyl-2-imino-5,5-diphenylimidazolidin-4-one		55	7.41-7.43 (m, 4H), 7.18-7.29 (m, 11H), 6.70 (s, 1H), 4.73 (s, 2H)	342.1	5.49 / 99
8g	3-benzyl-2-imino-5,5-bis(4-methoxyphenyl)imidazolidin-4-one		45	7.17-7.31 (m, 9H), 6.84 (d, *J*=8.8 Hz, 4H), 6.60 (s, 1H), 4.70 (s, 2H), 3.71 (s, 6H)	402.1	5.32 / 98

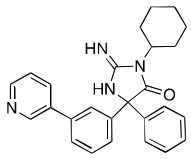
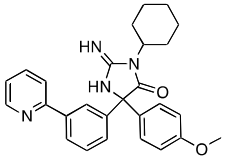
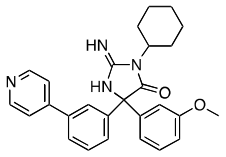
Ex.	IUPAC Name	Structure	Yield (%)	^{1}H NMR (400 MHz, DMSO-d_6) δ	LC-MS m/z $(M+H)^+$	HPLC t_R (min) / Purity (%)
8h	2-imino-5,5-bis(4-methoxyphenyl)-3-(2-phenylethyl)imidazolidin-4-one		50	7.18-7.21 (m, 9H), 6.81 (d, *J*=8.8 Hz, 4H), 6.57 (s, 1H), 3.71 (s, 6H), 3.58 (m, 1H), 2.78 (m, 1H)	416.1	6.13 / 96
8i	2-imino-5,5-bis(4-methoxyphenyl)-3-(3-phenylpropyl)imidazolidin-4-one		45	7.18-7.29 (m, 6H), 7.15 (d, *J*=8.4Hz, 1H), 7.12 (d, *J*=7.2Hz, 2H), 6.84 (d, *J*=8.4Hz, 4H), 6.60 (s, 1H), 3.70 (s, 6H), 3.51 (t, *J*=7.2Hz, 2H), 2.46 (m, 1H), 1.73 (m, 2H)	430.2	7.20 / 98
8j	2-imino-5,5-bis(4-methoxyphenyl)-3-(propan-2-yl)imidazolidin-4-one		50	7.25 (d, *J*=8.4Hz, 4H), 6.85 (d, *J*=8.4Hz, 4H), 6.44 (s, 1H), 4.11 (s, 1H), 3.71 (s, 6H), 1.31 (d, *J*=6.8Hz, 6H)	354.1	4.87 / 96
8k	3-tert-butyl-2-imino-5,5-diphenylimidazolidin-4-one		35	7.27-7.38 (m, 10H), 1.57 (s, 9H)	308.2	6.12 / 98
8l	3-cyclopropyl-2-imino-5,5-bis(4-methoxyphenyl)imidazolidin-4-one		55	7.27 (d, *J*=8.8 Hz, 4H), 6.83 (d, *J*=8.8 Hz, 4H), 6.50 (s, 1H), 3.70 (s, 6H), 2.50-2.55 (m, 1H), 1.92 (m, 2H), 0.71 (m, 2H)	352.1	4.53 / 99
8m	3-cyclobutyl-2-imino-5,5-bis(4-methoxyphenyl)imidazolidin-4-one		55	7.27 (d, *J*=8.8 Hz, 4H), 6.85 (d, *J*=8.8 Hz, 4H), 6.44 (s,1H), 4.38 (m, 1H), 3.71 (s,6H), 2.77 (m, 2H), 2.10 (m, 2H), 1.55-1.77 (m, 2H)	366.1	5.49 / 95
8n	3-cyclopentyl-2-imino-5,5-bis(4-methoxyphenyl)imidazolidin-4-one		50	7.25 (d, *J*=8.4 Hz, 4H), 6.85 (d, *J*=8.4 Hz, 4H), 6.47 (s, 1H), 4.21 (s, 1H), 3.71 (s, 6H), 1.94 (m, 2H), 1.74-1.79 (m, 4H), 1.50 (m, 2H)	380.1	6.14 / 96

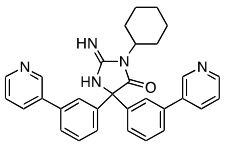
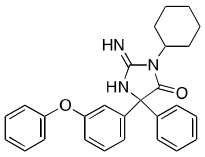
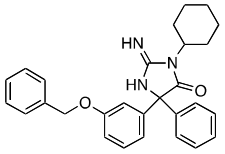
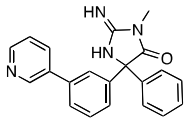
Ex.	IUPAC Name	Structure	Yield (%)	^{1}H NMR (400 MHz, DMSO-d_6) δ	LC-MS m/z $(M+H)^+$	HPLC t_R (min) / Purity (%)
8o	3-cyclohexyl-2-imino-5,5-diphenylimidazolidin-4-one		55	7.22-7.37 (m, 10H), 6.54 (s, 1H), 3.70 (m, 1H), 2.09 (m, 2H), 1.75 (m, 2H), 1.55(m, 3H), 1.08-1.28 (m, 3H)	334.1	7.34 / 98
8q	3-cycloheptyl-2-imino-5,5-bis(4-methoxyphenyl) imidazolidin-4-one		52	7.23 (d, *J*=8.8 Hz, 4H), 6.85 (d, *J*=8.8 Hz, 4H), 3.89 (m, 1H), 3.71 (s, 6H), 2.11 (m, 2H), 1.39-1.70 (m, 10H)	408.2	8.35 / 98
9a	2-imino-5,5-bis(4-methoxyphenyl) -3-(oxan-4-yl)imidazolidin-4-one		50	7.25 (d, *J*=8.0 Hz, 1H), 6.83 (d, *J*=8.4 Hz, 1H), 6.52 (s, 1H), 3.91 (m, 3H), 3.70 (s, 6H), 3.26 (m, 2H), 2.34 (m, 2H), 1.87 (m, 2H), 147 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) 158.6, 155.0, 135.5, 128.3, 113.7, 67.0, 55.5, 49.6, 29.2	396.1; HRMS calcd for $C_{22}H_{26}N_3O_4$ 396.1918; found 396.1917	4.44 / 99
9b	3-(4-hydroxycyclohexyl)-2-imino-5,5-bis(4-methoxyphenyl) imidazolidin-4-one		45	7.21(t, *J*=8.0, 8.4Hz), 6.99 (d, *J*=8.0Hz, 2H), 6.94 (s, 2H), 6.80 (d, *J*=8.0 Hz, 2H), 6.59 (s, 1H), 4.19 (m, 1H), 3.68 (s, 6H), 1.94 (m, 2H), 1.79 (m, 4H), 1.50 (m, 2H)	410.2	4.23 / 97
9c	2-imino-5,5-bis(4-methoxyphenyl) -3-(piperidin-4-yl)imidazolidin-4-one		~50	7.24 (d, *J*= 8.0 Hz, 4H), 6.84 (d, *J*= 8.0 Hz, 4H), 6.47 (s, 1H), 3.71 (s, 7H), 2.95 (m, 2H), 2.23 (t, *J*= 8.0 Hz, 2H), 2.16 (m, 2H), 1.43 (m, 2H)	395.1	9.66 / 99
9d	2-imino-5,5-bis(4-methoxyphenyl) -3-(1-methylpiperidin-4-yl)imidazolidin-4-one		52	7.24 (d, *J*=0.8 Hz, 1H), 6.83 (d, *J*=0.8 Hz, 1H), 6.46 (s, 1H), 3.71 (m, 1H), 3.70 (s, 6H), 2.79 (m, 2H), 2.35 (m, 2H), 2.14 (s, 3H), 1.87 (m, 2H), 145 (m, 2H)	409.2	5.66 / 95

Ex.	IUPAC Name	Structure	Yield (%)	^{1}H NMR (400 MHz, DMSO-d_6) δ	LC-MS m/z $(M+H)^+$	HPLC t_R (min) / Purity (%)
9e	tert-butyl 4-[2-imino-4,4-bis(4-methoxyphenyl)-5-oxoimidazolidin-1-yl]piperidine-1-carboxylate		55	7.23 (d, *J*=8.4 Hz, 1H), 6.83 (d, *J*=8.4 Hz, 1H), 6.52 (s, 1H), 4.02 (m, 2H), 3.86 (m, 1H), 3.70 (s, 6H), 2.70 (m, 2H), 2.14 (m, 2H), 1.52 (m, 2H) , 1.40 (s, 9H)	495.2	6.94 / 96
9f	3-(cyclohexylmethyl)-2-imino-5,5-bis(4-methoxyphenyl)imidazolidin-4-one		50	7.28 (d, *J*=8.4 Hz, 4H), 6.83 (d, *J*=8.4 Hz, 4H), 6.53 (s, 1H), 3.70 (s, 6H), 3.28-3.36 (m, 2H), 1.60 (m, 4H), 1.49 (m, 2H), 1.09 (m, 2H), 0.88 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) 181.1, 158.6, 155.7, 135.6, 128.3, 114.0, 55.4, 45.0, 36.7, 30.2, 26.3, 25.5	408.2; HRMS calcd for $C_{24}H_{30}N_3O_3$ 408.2282; found 408.2284	7.68 / 94
9g	2-imino-5,5-bis(4-methoxyphenyl)-3-(oxan-4-ylmethyl)imidazolidin-4-one		52	7.29 (d, *J*=8.8 Hz, 4H), 6.84 (d, *J*=8.4 Hz, 4H), 6.58 (s, 1H), 3.78 (m, 2H), 3.70 (s, 6H), 3.35 (m, 2H), 3.17 (m, 2H), 1.83 (s, 1H), 1.37 (m, 2H), 1.11-1.21 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) 158.5, 155.3, 135.5, 128.2, 113.7, 66.8, 55.4, 44.5, 34.4, 30.3	410.2; HRMS calcd for $C_{23}H_{28}N_3O_4$ 410.2074; found 410.2075	4.60 / 98
10a	3-cyclohexyl-2-imino-5,5-bis(3-methoxyphenyl)imidazolidin-4-one		55	7.20 (m, 2H), 6.78-6.98 (m, 6H), 6.56 (s, 1H), 3.71 (m, 1H), 3.70 (s, 6H), 2.08 (m, 2H), 1.74 (m, 2H), 1.52 (m, 3H), 1.10-1.30 (m, 3H)	394.2	6.99 / 93

Ex.	IUPAC Name	Structure	Yield (%)	^{1}H NMR (400 MHz, DMSO-d_6) δ	LC-MS m/z $(M+H)^+$	HPLC t_R (min) / Purity (%)
(±)-**10b**	3-cyclohexyl-2-imino-5-(4-methoxyphenyl)-5-phenylimidazolidin-4-one		42	7.26-7.34 (m, 7H), 6.85 (d, *J*= 8.8 Hz, 2H), 6.50 (s, 1H) ,3.71 (m,4H), 2.10 (m, 2H), 1.74 (m, 2H), 1.54 (m, 3H), 1.24 (m, 2H), 1.17 (m, 1H)	364.2	7.22 / 98
(±)-**10c**	3-cyclohexyl-2-imino-5-(3-methoxyphenyl)-5-phenylimidazolidin-4-one		45	7.19-7.37 (m, 6H), 6.93-6.98 (m, 3H), 6.80 (d, *J*=6.8 Hz, 1H), 3.70 (m, 1H), 3.68 (s, 3H), 2.09 (m, 2H), 1.75 (m, 2H), 1.51-1.59 (m, 3H), 1.07-1.35 (m, 3H)	364.1	7.17 / 97
10d	3-cyclohexyl-2-imino-5,5-bis[4-(trifluoromethoxy)phenyl]imidazolidin-4-one		50	7.42-7.46 (m, 4H), 7.26-7.31 (m, 4H), 6.83 (s, 1H), 3.72 (m, 1H), 2.06 (m, 2H), 1.74 (m, 2H), 1.56 (m, 3H), 1.10-1.30 (m, 3H)	502.1	9.25 / 98
10e	3-cyclohexyl-5,5-bis(4-ethoxyphenyl)-2-iminoimidazolidin-4-one		50	7.22 (d, *J*=8.4 Hz, 4H), 6.82 (d, *J*=8.4 Hz, 4H), 6.44 (s, 1H), 3.98 (m, 4H), 3.69 (s, 1H), 2.08 (m, 2H), 1.74 (m, 2H), 1.51-1.59 (m, 3H), 1.07-1.35 (m, 9H)	422.2	10.19 / 96
10f	3-cyclohexyl-2-imino-5,5-bis(4-methylphenyl)imidazolidin-4-one		45	7.20 (d, *J*=8.4Hz, 4H), 7.12 (d, *J*=8.4 Hz, 4H), 3.80 (m, 1H), 2.26 (s, 6H), 2.07(m, 2H), 1.73 (m, 2H), 1.58 (m, 3H), 1.07-1.31 (m, 3H)	362.2	11.32 / 90
10g	3-cyclohexyl-2-imino-5,5-bis(3-methylphenyl)imidazolidin-4-one		48	7.02-7.17 (m, 8H), 6.51 (s, 1H), 3.71 (m, 1H), 2.24 (s, 6H), 2.09 (m, 2H), 1.74 (m, 2H), 1.53 (m, 3H), 1.10-1.30 (m, 3H)	362.2	10.01 / 86

Ex.	IUPAC Name	Structure	Yield (%)	^{1}H NMR (400 MHz, DMSO-d_6) δ	LC-MS m/z $(M+H)^+$	HPLC t_R (min) / Purity (%)
10h	5,5-bis(4-chlorophenyl)-3-cyclohexyl-2-iminoimidazolidin-4-one		45	7.34-7.39 (m, 8H), 6.67 (s, 1H), 3.70 (m,1H), 2.02-2.10 (m, 2H), 1.73 (m, 2H), 1.56 (m, 3H), 1.09-1.29 (m, 3H)	402.1	16.97 / 97
10i	5,5-bis(3-chlorophenyl)-3-cyclohexyl-2-iminoimidazolidin-4-one		45	7.30-7.38 (m, 8H), 6.76(s, 1H), 3.71 (m, 1H), 2.06 (m, 2H), 1.74 (m, 2H), 1.51-1.59 (m, 3H), 1.07-1.35 (m, 3H)	402.1	13.77 / 97
(±)-**10j**	3-cyclohexyl-2-imino-5-(4-methoxyphenyl)-5-(3-phenylphenyl)imidazolidin-4-one		45	7.63 (s, 1H), 7.30-7.52 (m, 10H), 6.86 (d, *J*=8.4 Hz, 2H), 6.55(s, 1H), 3.71 (m, 4H), 2.10 (m, 2H), 1.75 (m, 2H), 1.51-1.59 (m, 3H), 1.07-1.35 (m, 3H)	440.2	13.30 / 98
(±)-**10k**	3-cyclohexyl-2-imino-5-(4-methoxyphenyl)-5-[3-(pyridin-3-yl)phenyl]imidazolidin-4-one		40	8.73 (s, 1H), 8.56 (d, *J*=1.6 Hz, 1H), 7.92 (m, 1H), 7.65 (s, 1H), 7.58 (m, 1H), 7.44-7.50 (m, 4H), 7.31 (d, *J*=8.8 Hz, 2H), 6.87 (d, *J*=8.8 Hz, 2H), 3.77(m, 1H), 3.70 (s, 3H), 2.10 (m, 2H), 1.74 (m, 2H), 1.51-1.59 (m, 3H), 1.07-1.35 (m, 3H)	441.2	7.61 / 97
(±)-**10l**	3-cyclohexyl-2-imino-5-(3-methoxyphenyl)-5-[3-(pyridin-3-yl)phenyl]imidazolidin-4-one		40	8.74 (s, 1H), 8.57 (d, *J*=3.6 Hz, 1H), 7.92 (d, *J*=8.0 Hz, 1H), 7.68 (s, 1H), 7.58 (d, *J*=6.8 Hz, 1H), 7.44-7.50 (m, 3H), 7.22 (t, *J*=7.6, 8.0 Hz, 1H), 7.02 (d, *J*=7.6 Hz, 1H), 6.97 (s, 1H), 6.81 (m, 1H), 6.63 (s, 1H), 3.71 (m, 1H), 3.68 (s, 3H), 2.10 (m, 2H), 1.74 (m, 2H), 1.55 (m, 3H), 1.10-1.30 (m, 3H)	441.2	7.63 / 97

Ex.	IUPAC Name	Structure	Yield (%)	^{1}H NMR (400 MHz, DMSO-d_6) δ	LC-MS m/z $(M+H)^+$	HPLC t_R (min) / Purity (%)
(±)-**10m**	3-cyclohexyl-2-imino-5-phenyl-5-[3-(pyridin-3-yl)phenyl]imidazolidin-4-one		40	8.74 (s, 1H), 8.57 (d, *J*=3.2 Hz, 1H), 7.91 (d, *J*=7.6 Hz, 1H), 7.69 (s, 1H), 7.58 (d, *J*=7.2 Hz, 1H), 7.41-7.50 (m, 6H), 7.30 (t, *J*=7.2, 7.6 Hz, 2H), 7.23 (d *J*=7.2Hz, 1H), 6.60 (s, 1H), 3.72 (m, 1H), 2.10 (m, 2H), 1.73-1.76 (m, 2H), 1.51-1.59 (m, 3H), 1.07-1.35 (m, 3H)	411.2	7.77 / 97
(±)-**10n**	3-cyclohexyl-2-imino-5-(4-methoxyphenyl)-5-[3-(pyridin-2-yl)phenyl]imidazolidin-4-one		48	8.64 (d, *J*=4.4 Hz, 1H), 8.17 (s, 1H), 7.82-7.87 (m, 3H), 7.29-7.34 (m, 5H), 6.86 (d, *J*=8.0 Hz, 2H), 6.55 (s, 1H), 3.71 (m, 4H), 2.10 (m, 2H), 1.74 (m, 2H), 1.51-1.59 (m, 3H), 1.07-1.35 (m, 3H)	441.2	7.40 / 96
(±)-**10o**	3-cyclohexyl-2-imino-5-(3-methoxyphenyl)-5-[3-(pyridin-4-yl)phenyl]imidazolidin-4-one		45	8.63 (d, *J*=6.0 Hz, 2H), 7.78 (s, 1H), 7.65 (d, *J*=7.6 Hz, 1H), 7.54 (d, *J*=6.0 Hz, 3H), 7.46 (t, *J*=7.6 Hz, 1H), 7.22 (t, *J*=8.0 Hz, 1H), 7.01 (d, *J*=8.0 Hz, 1H), 6.96 (d, *J*=2.0 Hz, 1H), 6.81 (dd, *J*=2.4, 8.0 Hz, 1H), 6.65 (s, 1H), 3.71 (m, 1H), 3.68 (s, 3H), 2.08-2.11 (m, 2H), 1.75 (m, 2H), 1.51-1.59 (m, 3H), 1.07-1.35 (m, 3H)	441.2	7.84 / 97

Ex.	IUPAC Name	Structure	Yield (%)	^{1}H NMR (400 MHz, DMSO-d_6) δ	LC-MS m/z $(M+H)^+$	HPLC t_R (min) / Purity (%)
10p	3-cyclohexyl-2-imino-5,5-bis[3-(pyridin-3-yl)phenyl]imidazolidin-4-one		40	8.75 (s, 2H), 8.56 (d, *J*=3.6 Hz, 2H), 7.93 (d, *J*=8.0 Hz, 2H), 7.74 (s, 2H), 7.44-7.60 (m, 10H), 6.69 (s, 1H), 3.74 (m, 1H), 2.11(m, 2H), 1.75 (m, 2H), 1.58 (m, 3H), 1.11-1.31 (m, 3H)	488.2	8.20 / 98
(±)-**10q**	3-cyclohexyl-2-imino-5-(3-phenoxyphenyl)-5-phenylimidazolidin-4-one		43	7.35-7.38 (m, 6H), 7.29 (m, 2H), 7.12-7.27 (m, 2H), 6.99 (d, *J*=7.6 Hz, 2H), 6.93 (d, *J*=8.4 Hz, 2H), 6.56 (s, 1H), 3.68 (m, 1H), 2.07(m, 2H), 1.73 (m, 2H), 1.56 (m, 3H), 1.07-1.31 (m, 3H)	462.2	14.63 / 100
(±)-**10r**	5-[3-(benzyloxy)phenyl]-3-cyclohexyl-2-imino-5-phenylimidazolidin-4-one		42	7.20-7.39 (m, 12H), 6.93(d, *J*=8.0 Hz, 2H), 6.52 (s, 1H), 5.05 (s, 2H), 3.69 (m, 1H), 2.07-2.10 (m, 2H), 1.73-1.76 (m, 2H), 1.51-1.59 (m, 3H), 1.07-1.35 (m, 3H)	440.2	13.42 / 98
(±)-**11**	2-imino-3-methyl-5-phenyl-5-[3-(pyridin-3-yl)phenyl]imidazolidin-4-one		30	8.75 (s, 1H), 7.60 (dd, *J*= 1.6, 4.8 Hz, 1H), 7.93 (d, *J*= 8.0 Hz, 1H), 7.76 (s, 1H), 7.42-7.59 (m, 6H), 7.20-7.32 (m, 3H), 6.69 (s, 1H), 2.99 (s, 3H)	343.1	4.58 / 98

cLogP Calculations

cLogP values were calculated using the JChem calculators from ChemAxon (http://www.chemaxon.com)

In vitro Antimalarial Assays (3D7 and Dd2)

In vitro antimalarial activity was determined by a malaria SYBR Green I-based fluorescence (MSF) method described previously by Smilkstein et al. (2004, Antimicrob. Agents Chemother. 48:1803–1806) with slight modification (Winter, et al. 2006. Exp. Parasitol. 114:47–56.). Stock solutions of each test drug were prepared in DMSO at a concentration of 20 mM. The drug solutions were serially diluted with culture medium and distributed to asynchronous parasite cultures on 96-well plates in quadruplicate in a total volume of 100 µl to achieve 0.5% parasitemia with a 2% hematocrit in a total volume of 100 µl. The plates were then incubated for 72 h at 37°C. After incubation, 100 µl of lysis buffer with 0.2 µl/ml SYBR Green I was added to each well. The plates were incubated at 37°C for an hour in the dark and then placed in a 96-well fluorescence plate reader (Spectramax Gemini- EM; Molecular Diagnostics) with excitation and emission wavelengths at 497 nm and 520 nm, respectively, for measurement of fluorescence. The 50% inhibitory concentration (IC_{50}) was determined by nonlinear regression analysis of logistic dose-response curves (GraphPad Prism software). Antimalarial potency of compounds was determined by this technique for both *Plasmodium falciparum* 3D7 (CQ-sensitive) and Dd2 (multi-drug resistant) strains.

Plasmepsin-2 (PM-II; PM-2) and Plasmpesion-4 (PM-IV; PM-4) Enzyme Inhibition Assays

Plasmepsin-2 (PM-2; Plm II) and Plasmepsin-4 (PM-4; Plm IV) expression and purification were performed following the published protocols (Istvan ES and Goldberg DE, 2005). The final purified protein was activated by diluting the protein to 0.3mg/ml in activating buffer (0.1M citrate pH 4.5,0.1% Tween-20, 50mM dithiothreitol) and incubated at room temperature for 40 min, then the activated enzyme was diluted in assay buffer (50mM sodium acetate pH 4.7, 0.01% Tween-20). The enzymatic inhibition reaction was performed in 384 well plates with a total volume of 20 µl. 10 µl of diluted PM-2 or PM-4 enzyme was added to the 384-well plate except blank wells (blank wells add 10 µL of assay buffer) and 20 nl of serials of diluted 1000 × compounds were added to the wells with 520 Echo® Liquid Handling System (Labcyte Inc.). 10 µl PM-2 peptide substrate (AnaSpec, Cat#, 62050) with assay buffer was then added to final

concentration of 20 µM to start the reaction. After incubation the reaction at room temperature for 60 min , the fluorescence intensity at Ex/Em= 360nm/535nm was measured using EnVision multilabel plate reader (Perkin-Elmer). The IC_{50} values were obtained using Graph Pad Prism 4.

Plasmepsin-5 (PM-V) Enzyme Assay

GFP conjugated PM-V was isolated based on the method described in Russo *et al.*[2] from a pellet of P. *falciparum* (DC6) and anti-GFP (Invitrogen A11120,3E6) antibody. The immune complex was captured on protein A agarose and washed exhaustively with ice cold PBS. The agarose beads were suspended in 200 µl of PBS with 2mM dithiothreitol (DTT).

The assay was run in black, small volume 384 well plates (Greiner) containing 5 µl of agarose bead suspension plus 5µl of a compound dose response diluted to 4% DMSO in assay buffer (50mM Tris-Maleate pH 6.45, 50mM NaCl, 0.05% Triton X-100 plus 2mM DTT) . To this, 10µl of 100µM quenched PEXEL substrate (DABCYL-GNKRTLAQKQG–EDANS) in assay buffer was pipetted with gentle aspiration mixing. The fluorescence intensity (335ex/490em) of each well was monitored kinetically (15min intervals) using a Tecan Safire II plate reader heated to 37°C. A background reaction was carried out using a mutated PEXEL substrate (DABCYL-GNKATAAQKQG–EDANS) that was demonstrated in Boddey *et al.*[3] to not be cleaved by PM-V. The IC_{50} was calculated from an endpoint analysis at 75 min, plotting the signal minus background for each compound concentration using GraphPad Prism.

β-Secretase (BACE1), Cathepsin D, and Cathepsin E Enzymes Inhibition Assays

The recombinant human BACE1, Cathepsin D, and Cathepsin E enzymes were purchased from R&D Systems (catalog numbers are 931-AS, 1014AS and 1294AS, respectively). The enzymatic inhibition activities Assays were performed in a 384-well plate format using the fluorescence resonance energy transfer (FRET) assay. The concentration of BACE1 and Cathepsin D were 20 ng/µl, and 1 ng/µl of Cathepsin E was used. BACE1 was activated by incubation in assay buffer (100mM sodium acetate pH 4.0) for 15 min at room temperature;

CatD and CatE were activated by incubation in assay buffer (0.1 M NaOAc, 0.2 M NaCl, pH 3.5) at room temperature for 30 min. Subsequently, the rhBACE-1 substrate (R&D Systems, catalog# ES004), the Cathepsin D and Cathepsin E substrate ((R&D Systems, Catalog # ES001) were added accordingly to final concentration 20uM to initiate the reaction. After 60 min incubation at room temperature, the time-resolved fluorescence at Ex/Em= 360nm/460nm was measured on an EnVision multilabel plate reader (Perkin-Elmer). The analytical software, GraphPad Prism 5.0 (GraphPad Software, Inc., USA) was used to generate IC_{50} values via non-linear regression analysis.

Cytochrome P450 inhibitory assay

The cytochrome P450 inhibitory potentials of compounds for human recombinant CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A were determined using Vivid CYP450 blue screening kits (Invitrogen,USA). All of the procedures were performed according the instruction provided by the manufacturer. Briefly, Serials of diluted compounds were incubated the vivid CYP450 reaction systems including CYP450 BACULOSOMES with different recombinant human CYP45o isozymes and the appropriate vivid CYP450 substrates, and rabbit NADPH-P450 reductase, and the regeneration system. Ketoconazole was used as reference CYP450 inhibitor. After 30 minutes incubation at room temperature, the fluorescence was measured with an excitation at 400 nm and an emission at 460 nm using Envision 2104 multi-label Reader (Perkin Elmer, USA). The IC50 values were calculated by nonlinear regressions using GraphPad Prism 5.

HepG2 Cytotoxicity Assay

HepG2 cells (ATCC Cat. No. HB-8065) were maintained in DMEM supplemented with 10% fetal bovine serum and L-glutamine. Cells were grown at 37ºC and 5% CO_2 on flasks coated with poly-d-lysine. To assess compounds for potential cytotoxic properties cells were plated at 10,000 cells per well on 96-well poly-d-lysine plates at 10,000 cells per well. HepG2 cells were

allowed to adhere for at least 4 hours prior to application of test compounds. Cells were incubated with test compounds for 72 hours before measuring cellular viability. Cellular viability was measured using PrestoBlue® Cell Viability Reagent (Life Technologies). Briefly, 11ul of 10X PrestoBlue reagent was added to 100ul in each assay well. Cells were incubated at 37ºC and 5% CO_2 for 30 minutes prior to reading on Tecan Safire^2, excitation at 560nm, 10 nm bandwidth and emission at 590 nm, 10 nm bandwidth. GraphPad Prism 5.0 (GraphPad Software, Inc., USA) was used to generate IC_{50} values via non-linear regression analysis.

MLM, RLM and HLM Assays

In this protocol, the metabolic stability of compounds at 1 µM was determined in Mouse Liver Microsome (MLM), rat liver microsomes (RLM) or human liver microsomes (HLM). Each test compound was incubated in an aqueous reaction mixture (0.6mL total volume) consisting of animal or human liver microsomal protein and NADPH(1.2mM) in the presence of 100mM potassium phosphate buffer (pH 7.4), and 3.3mM $MgCl_2$. The final concentration of each substrate was 1 µM, and the microsomal protein concentration was 0.25 µM(concentration of substrate and microsome are subject to change according to individual case). After incubation at 37◦C for a specific time period (0, 5, 10, 20 and 30 min), the reaction was terminated by the addition of 200 µL ice cold acetonitrile containing internal standard(100ng/mL) after100 uL aliquots of reaction mixture was removed. The quenched reaction mixtures were centrifuged at 3200 rpm for 5 min, and 100 µL of the supernatant were transferred to 96-well deep plate. The samples in 96 well plate were analyzed by LC-MS/MS using an Applied Biosystems-Sciex model API 3000 mass spectrometer. The data were analyzed using the following equations.

Compound response = Analyte Area/IS Area

Relative concentration = ($T_{response}$ / $T_{0\ response}$) * 100%

Half life($T_{1/2}$)(min)=0.693/ Kdep

V(µL/mg)=volume of incubation(µL)/protein in the incubation(mg)

Clearance(CL)(µL/min/mg protein)=V×0.693/ $T_{1/2}$

Human Plasma Protein Binding (hPPB) Assay

Human Plasma Protein Binding (hPPB) of test compounds was measured by rapid equilibrium dialysis device using the RED Device Inserts. In the reaction of 5h, the drug concentration of the sample chamber and buffer chamber can be determined, through which we can calculated hPPB of test compounds. RED Device Inserts and Single-Use RED Base Plate were purchased from the Linden Bioscience, Woburn, MA (Taiwan, Thermo scientific). Human plasma was obtained from one healthy volunteer. Pooled human plasma from healthy individuals in this study was obtained from the Southern Medical University (China) and was stored frozen at −20 ℃ until use. SHZ-88A Reciprocating thermostatic oscillator was purchased from Taicang City Experimental Equipment Co. (China). Chlorpromazine (>99% in purity,positive control) and Phenacetin (>99% in purity, internal standard, IS) were all purchased from the Sigma Chemical Co. (China). Chlorpromazine HCl was used as a positive control. An aliquot (300 μL) of plasma was accurately added into the sample chamber, and 6 μl compounds solution (500 μM, 50%DMSO) was added (n=3). An aliquot (500 μL) of Dulbecco solution was added into the buffer chamber. These solution were all oscillated for 5 hours (37 ℃, 90 rpm) . Then, 50 μL solution in sample chamber was removed, 50 μL Dulbecco solution was added, and mixed. Also, 50μL solution in buffer chamber was removed , 50 μL plasma was added, and mixed. 200 μ L ACN (including internal Standard, 100 ng/mL) were added separately, oscillated, mixed, and centrifuged (20 min, 15000g). An aliquot (50 μL) of the supernatant was accurately transferred to a clean 1.5mL test tube and 100 μL H2O was added, mixed. 10 μL of mixture was separately injected into LC-MS system for determination of the drug concentration in the sample chamber and buffer chamber. The data were analyzed using the following equation: % hPPB =[1-(Concentration $_{\text{buffer chamber}}$/Concentration $_{\text{plasma chamber}}$)] *100% (Barre, J., Chamouard, J.M., Houin, G., Tillement, J.P., 1985. Equilibrium dialysis, ultrafiltration and ultracentrifugation compared for determining the plasma protein binding characteristics of valproic acid. Clin. Chem. 31, 60–64.)

Rat Pharmacokinetic (PK) Analysis

Male SD rats, weighing 180-220g (Southern China Medical University,China) were utilized for the studies. The protocol was approved by the Institutional Animal Care and Use Committee at GIBH. Animals were maintained on standard animal chow and water ad libitum, in a climate controlled room(23±1℃, 30–70% relative humidity, a minimum of 10 exchanges of room air per hour and a 12-h light/dark cycle) for one week prior to experiments. The test compound was dissolved in suitable solvent. Pharmacokinetic properties were determined following i.v. and oral administration. Animals were randomly distributed into two experimental groups (n = 4). The oral groups were given 5 mg/kg of the test compound by gastric gavage. The other group was dosed by injection into the tail vein (1mg/kg). After single administration, whole blood samples (100-200 μL) were obtained from the orbital venous plexus at the following time points after dosing: 5,10, 30 min and 1, 2, 3, 4, 6, 8,11 and 24 h(p.o.); 2,10, 30 min and 1, 2, 3, 4, 6, 8, 11 and 24 h (i.v.).Whole blood samples were collected in heparinized tubes. The plasma fraction was immediately separated by centrifugation (8,000 rpm, 6 min, 4 °C) and stored at −20 °C until LC-MS analysis. The rats were humanely euthanasia by carbon dioxide 24 hours after experiment without pain.

Plasma sample analysis. Standard curve sample preparation: The compound was dissolved in DMSO at a concentration of 2mg/ml and diluted with 50% methanol solution to series concentration as follow:20, 50, 100, 500, 1000, 2000, 4000, 6000, 12000, 40000ng/ml. 10μl series concentration solution and 50μl blank plasma were added to 1.5ml tube and vortex for 3min, then 150μl acetonitrile containing internal standard were added and vortex for 5min, finally spin tube in centrifuge at 16000g for 40min at 4°C. **Plasma sample preparation:** The plasma samples were prepared using protein precipitation method.10μl 50% methanol water solution and 50μl plasma samples were added to 1.5ml tube and vortex for 3min, then 150μl acetonitrile containing internal standard were added and vortex for 5min, finally spin tube in centrifuge at 16000g for 40min at 4°C. **LC/MS/MS analysis:** After centrifuge, 100μl supernatant was transfer to the 96 well plate and analyzed by LC-MS/MS using an Applied Biosystems-Sciex model API 3000 mass spectrometer. The pharmacokinetics parameters were

calculated by analyzing the compound concentration in plasma samples using the pharmacokinetic software DAS.2.0

In vivo Antimalarial Efficacy Suppressive Assay

In vivo antimalarial activity was determined for compounds against the rodent *Plasmodium chabaudi ASS* and *ASCQ* strains according to the 4-day suppressive test (Peters, 1975). Briefly, NIH mice (n = 8 per group) were inoculated i.p. with $2x10^7$ parasitized (*Plasmodium chabaudi ASS* or *ASCQ*) red blood cells. Thereafter, the compounds were administered orally to the animals once daily at 4h, 24h, 48h and 72h post inoculation. Groups (n = 6) including a vehicle control and chloroquine (CQ) as a reference drug were included. Parasitemia levels were determined on the day following the last treatment (Day 4).

The Institutional Animal Care and Use Committee at the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, reviewed and approved the animal use in these studies. The animal care and use program, run entirely according to Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALACi) standards, is applying for AAALACi accreditation. The license for using laboratory animals is issued by Guangdong Laboratory Animal monitoring Institute. The Office of Laboratory Animal Welfare (OLAW) has recently re-activated the Animal Welfare Assurance for this institution (OLAW identification number A5748-01).

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^{1}H and ^{13}C NMR Spectra for compound 8p (CWHM-117; XJ-105062)