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Evaluation of spiropiperidine hydantoins as a novel class of antimalarial agents

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Abstract

Given the rise of parasite resistance to all currently used antimalarial drugs, the identification of novel chemotypes with unique mechanisms of action is of paramount importance. Since *Plasmodium* expresses a number of aspartic proteases necessary for its survival, we have mined antimalarial datasets for drug-like aspartic protease inhibitors. This effort led to the identification of spiropiperidine hydantoins, bearing similarity to known inhibitors of the human aspartic protease β-secretase (BACE), as new leads for antimalarial drug discovery. Spiropiperidine hydantoins have a dynamic structure-activity relationship profile with positions identified as being tolerant of a variety of substitution patterns as well as a key piperidine N-benzyl phenol pharmacophore. Lead compounds **4e** (CWHM-123) and **12k** (CWHM-505) are potent antimalarials with IC_{50} values against *Plasmodium falciparum* 3D7 of 0.310 μM and 0.099 μM, respectively, and the former features equivalent potency on the chloroquine-resistant Dd2 strain.

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Author Contributions

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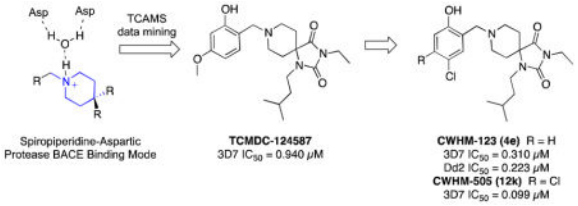
Remarkably, these compounds do not inhibit human aspartic proteases BACE, cathepsins D and E, or *Plasmodium* plasmepsins II and IV despite their similarity to known BACE inhibitors. Although the current leads suffer from poor metabolic stability, they do fit into a drug-like chemical property space and provide a new class of potent antimalarial agents for further study.

Graphical Abstract



Keywords

aspartic protease inhibitors; spiropiperidine hydantoins; antiplasmodial; antimalarial

1. Introduction

Malaria is caused by the parasite *Plasmodium*. In 2013, there were approximately 198 million cases of malaria leading to ~584,000 deaths, being particularly deadly to young children in sub-Saharan Africa.[1] *P. falciparum*, the most lethal species, has developed varying degrees of resistance to all currently used antimalarial drugs.[2–5] Approaches to combat parasite resistance include combination of antimalarial drugs as standard treatment regimens, as well as identification of new antimalarial drugs with unique mechanisms of action that can be combined with existing antimalarial drugs.

Plasmodium expresses a number of aspartic proteases necessary for its survival, including essential aspartic proteases Plasmepsin V (PMV or PM-5) and signal peptide peptidase (*Pf*SPP).[6–11] While a number of potent peptidomimetic inhibitors of *Plasmodium* aspartic proteases have been identified,[7, 12–14] we have focused on repurposing classes of drug-like aspartic protease inhibitors developed by the pharmaceutical industry for human aspartic proteases such as β-secretase (BACE)[15, 16] or renin.[17]

We have hypothesized that maintaining core structural motifs known to bind the aspartate residues in the active site may allow identification and optimization of novel classes of antimalarial compounds. Accordingly, we mined the Tres Cantos Anti-Malarial dataset (TCAMS) representing thousands of compounds[18] for drug-like aspartic protease inhibitors. For example, we recently reported our identification and initial optimization of aminohydantoins as novel antimalarial compounds with selectivity for *Plasmodium* and *in vivo* antimalarial efficacy (e.g., **CWHM-117**) originating from BACE inhibitor **1** and database hit **TCMDC-136879** (Figure 1a).[19]

Spiropiperidine-containing compounds such as **2** and **3** have been reported as non-peptidomimetic BACE inhibitors[16, 20–22] and represent a novel scaffold for development of new antimalarial aspartic protease inhibitors (Figure 1b). The reported x-ray crystal structure

of **2** (3FKT)[16] demonstrates the mechanism by which the protonated piperidine nitrogen forms a salt bridge with a water molecule in the active site. Similarly, other related piperidine and pyrrolidine BACE, renin and HIV protease inhibitor crystal structures demonstrate similar binding modes,[17, 23] leading us to hypothesize that the spiropiperidine scaffold may be an appropriate core for mining antimalarial phenotypic screening databases. Substructure-based searching of the TCAMS revealed a single hit, **TCMDC-124587** (**4a**), with a reported XC_{50} of 0.840 μM. Given its modest molecular weight, favorable CLogP, and submicromolar antimalarial potency, an effort to validate this hit and evaluate the potential of this class of spiropiperidines as antimalarials was initiated.

2. Results and discussion

2.1. Validation of hit and initial SAR

Searches of commercially available compound databases revealed that **TCMDC-124587** and closely-related analogs could be purchased from ChemBridge. Most commericially-available compounds were derivatized at the R^8 position. Two iterations of sets of six spiropiperidines each, including **TCMDC-124587**, were purchased and evaluated for inhibition of parasite growth in *P. falciparum* 3D7-infected red blood cells. Key structure-activity relationships are shown in Figure 2. Of foremost importance, **4a** was found to have similar 3D7 potency (IC_{50} = 0.940 μM) as reported in the screening dataset. Substituent position was found to be important. For example, moving the methoxy group from the 4′- to the 3′- or 5′-positions resulted in 6-fold loss or 2-fold improvement in potency, respectively (**4b,c**). While deletion of the methoxy group (**4d**) did not have a significant impact on potency, replacement with chlorine (**4e**) gave about a five-fold improvement in potency. Most striking is the dependence of potency on the presence of the phenol moiety. Capping the phenol with a methyl group (**4g**) or deletion (**4f,h**) led to 8- to 60-fold losses in potency.

The antimalarial activity of lead compound was determined to not be due to general cytoxicity (HepG2 72 h cytoxicity IC_{50} = 37 μM), having a selectivity index of >100-fold. We were further encouraged by identification of **4e** in the Novartis-GNF antimalarial screening hit collection (GNF-Pf-5345, reported EC_{50} = 0.349 μM), although only a few related compounds were present in this collection.[24] These data, along with the demonstration of a discrete SAR, encouraged us to investigate this class further by resynthesizing lead compound **4e** and broadening the SAR.

2.2. Synthesis

The synthesis of **4e** and related analogs is shown in Scheme 1. The spirohydantoin core **6** was prepared as previously described.[25] The R^3 alkyl group was then incorporated by simple alkylation with potassium carbonate. Subsequent alkylation with sodium hydride and R^1X, followed by deprotection of the BOC group afforded intermediate **9**. Finally, reductive amination provided R^8 analogs such as **4e**, **10a–b**, **10d–e, 10g–i, 11a–d** and **12a–u**. Additionally, some R^8 analogs were prepared from **8** standard amide coupling or alkylation conditions.

Sterically hindered or otherwise incompatible R^1 analogs were prepared instead by the approach reported by Carrera and Garvey[26] shown in Scheme 2. In brief, the primary R^1 amine is condensed with ketone **13** to furnish α-amino nitriles **14**. Condensation with potassium cyanate and ring closure gives access to mono-substituted hydantoins **15**. Subsequent alkylation, deprotection and reductive amination provided the final compounds **10c** and **10f**.

2.3. Structure-Activity Relationship Studies

Structure-activity relationships in the *Pf*3D7-infected red blood cell assay for R^1 and R^3 derivatives are shown in Figures 3 and 4. Resynthesized **4e** was found to have a similar IC_{50} value as the commercial lot and was used a reference compound for subsequent SAR studies. Keeping R^8 constant as the 5′-chloro-2′-hydoxybenzyl group, R^1 and R^3 were varied independently. R^1 proved tolerant of modification provided that the substituents remained suitably large and flexible. For example, replacement of the isopentyl (**4e**) with hydrogen (**10a**) or ethyl (**10b**) led to a 20-fold or greater reduction in potency. Similarly, substitution with a phenyl ring (**10d**) proved detrimental to potency while cyclopentyl (**10c**) was well tolerated. This is presumably due to the rigidity of the phenyl ring since extension with one (**10e**) or two (**10f**) methylene linkers were roughly equipotent with the isopentyl group. Benzyl analogs with electron donating (**10g**) or withdrawing (**10h,i**) groups were tolerated with some modest effects on potency.

SAR at the R^3 position also showed tolerance for a variety of lipophilic groups (Figure 4). Deletion of the ethyl group (**11a**) led to a dramatic loss in activity. Conversely, the ethyl group could be replaced with larger groups such as isopropyl (**11b**), which improved potency three-fold, or CH_2-pyridyl (**11c**) and $(CH_2)_3$-pyridyl (**11d**) with only modest two-three-fold losses of potency.

Given the relative flexibility in SAR in the R^1 and R^3 positions, we elected to focus on expanding the more sensitive SAR in the R^8 position (Figure 5). Moving the phenolic hydroxyl group to the 3′- or 4′-positions proved to be increasingly detrimental to 3D7 potency (**12a,b**) but not as dramatically as deletion of the hydroxyl group (**4f**). In contrast, the chlorine atom could be replaced by a variety of substituents. For example, while F is not an equivalent replacement (**12c**), bromo, methyl, and phenyl replacements are all well tolerated (**12d,e,f**).

Positioning of the chlorine atom was found to be important for optimal 3D7 potency for this series. 6′-Chloro analog **12g** was found to be essentially equipotent to the 5′-chloro **4e**. In contrast, the 3′-chloro and 4′-chloro analogs (**12h,i**) were three- to seven-fold less potent. 3′, 5′-Dichloro and 5′,6′-dichloro analogs (**12j,l**) were equipotent to the 5′-chloro compound **4e**. Interestingly, with an IC_{50} of 0.090 μM, 4′,5′-dichloro analog **12k** proved to be the most potent compound we identified in this series.

To further refine our understanding of the importance of the basic amine and benzylic phenol functionalities, we prepared amide analog **12m**. The lack of 3D7 potency of analog **12m**, a direct comparator to lead **4e,** demonstrates the importance of basic amine to the anti-*plasmodium* pharmacophore. This result is consistent with the hypothesis that these

compounds may be inhibiting an aspartic protease, similar to their BACE-inhibiting predecessors **2** and **3**. However, the basic amine functionality alone is not sufficient for potency as demonstrated by deletion analog **12n** and phenol replacements and isosteres (**12o-u**) which were not efficacious up to 10 μM. The only phenol replacement to give a hint of potency was indole **12u**.

2.4. Inhibition of Aspartic Proteases, Cytotoxicity and Mechanism of Action

We evaluated twelve of these compounds for inhibition against a panel of aspartic proteases: human proteases BACE-1, cathepsin D (CatD) and cathepsin E (CatE), as well as plasmodium proteases plasmepsin II and IV (PM-II and PM-IV). Remarkably, all twelve compounds had no inhibitory activity on these aspartic proteases up to 10 μM. Our previously described series,[19] the aminohydantoins, represented by **CWHM-117**, are also not potent against human aspartic proteases, but exhibit low nanomolar inhibition of plasmepsins II and IV. This selectivity result is encouraging from a toxicology and drug discovery standpoint but calls into question whether these compounds are acting through an aspartic protease mechanism.

In addition to compound **4e**, compounds **12f**, **12h**, and **12k** were evaluated for cytotoxicity in HepG2 cells. All three compounds exhibited IC_{50} values >50 μM, corresponding to a selectivity index of greater than 500 for **12k**.

The mechanism of action of these compounds is currently unknown. Notably, spiropiperidine **4e** is equipotent against both chloroquine-sensitive (3D7) and multidrug-resistant (Dd2) strains of *P. falciparum* (3D7 IC_{50} = 0.31 μM; Dd2 IC_{50} = 0.22 μM). The Dd2 strain is resistant to antimalarial drugs chloroquine, quinine, pyrimethamine, and sulfadoxine.[27] Thus, the mechanism of action of these spiropiperidine hydantoins appears to be unique and is not due to pathways affected by these existing agents.

2.5. Pharmacokinetic Profile

Finally, we evaluated the pharmacokinetic (PK) properties of this novel series of antimalarials. A subset of analogs were assessed for metabolic stability in mouse, rat and human liver microsome assays (MLM, RLM and HLM, respectively). Figure 6 depicts relationships between antimalarial activity, metabolic stability and lipophilicity. All of the compounds evaluated herein fall within generally acceptable lipophilicity ranges for druglike compounds (Fig. 6a). Not surprisingly, there is a correlation between lipophilicity and 3D7 potency with the more lipophilic compounds being generally more potent.

Most of the compounds evaluated have poor metabolic stability in liver microsomes with half-lives typically less than 10 minutes, regardless of species (Fig 6b). A handful of analogs, however, are metabolically stable (**12n,o,r**) but are not potent in the 3D7 assay. These stable compounds all have CLogP values of approximately 1.0 and do not contain an N-benzyl group. This data suggests that the metabolic stability for this series of compounds requires either compounds with low CLogP (less than 2), non-benzylic amines or a combination of both features.

Lead compound **4e** was also evaluated in vivo in a rat PK experiment to determine if in vitro liver microsome assays are predictive of in vivo PK properties. Compound **4e** has short half-lives in vitro (MLM, RLM and HLM $t_{1/2}$ = 4.0, 3.0 and 9.0 min, respectively) and in vivo (rat PK $t_{1/2}$ = 0.28 h, CL = 152 mL/min/kg, Vd = 3.7 L/kg and F = 10%). However, the modest oral bioavailability is encouraging.

3. Conclusions

We have identified spiropiperidine hydantoins as a novel series of antimalarial compounds with oral bioavailability but short half-lives. We have explored structure-activity relationships for the three pendant groups and found that the R^1 and R^3 positions tolerate a variety of functionality, suggesting that modulation of these positions should allow modulation of physiochemical properties without detrimental effects on potency. However, the R^8 benzylic phenol was found to be very sensitive to modification. We were able to demonstrate ~10-fold improvement in antimalarial activity through addition of chlorine atoms in the 4′ and 5′ positions, but replacement of the phenol or basic amine functionality resulted in dramatic losses in antimalarial potency. Unfortunately, this dependency on a benzylic amine for potency results in a series of compounds that face significant metabolic stability challenges that will need to be overcome in lead optimization. Progress towards understanding the mechanism of action and identification of more metabolically stable compounds will be reported in due course.

4. Experimental

4.1 General

Commercially available reagents and solvents were used without further purification unless stated otherwise. LC-MS analyses were performed on an Agilent 1100 or 1200HPLC/MSD electrospray mass spectrometer in positive ion mode with scan range was 100–1000d. Preparative normal phase chromatography was performed on a Biotage SP1 with prepacked Biotage or Varian silica gel cartridges. Preparative reverse phase HPLC was performed on a Shimadzu LC-20AP or Biotage SP1 equipped with a C18 column and a methanol/water or acetonitrile/water/0.05% TFA gradient. The purity of tested compounds was ≥95% as determined by HPLC analysis conducted on an Agilent 1100 or 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.

4.2. Purchased Compounds

Compounds **4a–g** and **11c–d** were purchased from ChemBridge (www.hit2lead.com). Compound **4e** was resynthesized as described in section 4.3.

4.3. Synthesis of CWHM-123 (4e)

***tert*-Butyl 2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (6)—**A solution of 11.25 g KCN in 25 mL water was added drop wise to a suspension of 16.24 g 1-

Bocpiperidone (Oakwood; 80 mmol) and 16.9 g ammonium carbonate in 45 mL water and 55 mL MeOH. During the addition, everything dissolved. The reaction flask was closed up with a balloon. After several hours a ppt began to form. The mixture was stirred at room temp over the weekend. After 72 h, the thick reaction mixture was filtered, washed with small portions of water and dried to give the desired product as a white solid: 16.1 g, 59.8 mmol, 75% yield. HPLC purity >98%. ES-MS *m/z* 292 (M+Na).

***tert*-Butyl 3-ethyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate (7a)—**A mixture of **6** (3.03 g, 11.3 mmol) and potassium carbonate in DMF (30 mL) was treated with ethyl iodide (1.0 mL, 12.4 mmol) dropwise at room temp. After 5 h, the reaction was diluted with EtOAc, washed with brine, dried over sodium sulfate, filtered and concentrated to give the crude product. The crude solid was recrystallized from EtOAc/heptane to give the title compound as a white solid (2.53 g, 8.51 mmol, 75% yield). HPLC purity >98%. ES-MS *m/z* 298 (M+H).

3-ethyl-1-isopentyl-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (12n)—7a (3.16 g, 10.6 mmol) was dissolved in 30 mL DMF (anhydrous) and was treated with NaH (60% disp. in mineral oil, 483 mg, 12.1 mmol) at room temp. After 15 min of stirring, 1-iodo-3-methylbutane (1.7 mL, 12.9 mmol) was added dropwise. After 5 days, the reaction was quenched with water and partitioned between EtOAc and satd ammonium chloride. The organic layer was washed with satd ammonium chloride twice, and was dried with sodium sulfate, filtered and concentrated. The crude product was purified by silica gel chromatography (0–30% EtOAc/hexane) to give 3.1 g of *tert*-butyl 3-ethyl-1-isopentyl-2,4-dioxo-1,3,8-triazaspiro[4.5]decane-8-carboxylate as a clear oil. The oil was then dissolved in 10 mL of diethyl ether, and 35 mL of 1 M HCl was added. The reaction was stirred at room temp for 48 h. The precipitate was then filtered and washed with diethyl ether, yielding the title compound as a white, powdery HCl salt. (2.13 g, 7.02 mmol, 66% yield). HPLC purity > 90%. ES-MS m/z 268 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.26 (br. s., 1 H), 3.39 (q, J=7.2 Hz, 2 H), 3.32 (d, J=6.9 Hz, 4 H), 3.18 (t, J=1.0 Hz, 2 H), 2.36 (dt, J=14.1, 9.2 Hz, 2 H), 1.86 (d, J=14.4 Hz, 2 H), 1.55 (spt, J=1.0 Hz, 1 H), 1.45 (q, J=1.0 Hz, 2 H), 1.08 (t, J=1.0 Hz, 3 H), 0.91 (d, J=1.0 Hz, 6 H).

8-(5-chloro-2-hydroxybenzyl)-3-ethyl-1-isopentyl-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride (CWHM-123; 4e)—A mixture of **299** hydrochloride (200 mg, 0.658 mmol) and 5-chloro-2-hydroxybenzaldehyde (113 mg, 0.724 mmol) in DCE (2 mL) was treated with sodium triacetoxyborohydride (279 mg, 1.32 mmol) followed by DMF (1 mL). The suspension was stirred at room temp overnight. The reaction was quenched with water, acidified with TFA, concentrated and purified by reverse phase HPLC (10 to 70% acetonitrile/water/0.05% TFA). Excess acetonitrile was removed under vacuum and the resultant aqueous solution was neutralized with saturated sodium bicarbonate, extracted with EtOAc, washed with brine, dried over sodium sulfate and concentrated to give a white foam (191 mg). The white foam was dissolved in diethyl ether and treated with 1 N HCl in diethyl ether (0.6 mL). The white ppt was filtered, washed with ether and dried to give the title compound a white HCl salt (194 mg, 0.436 mmol, 66% yield). HPLC purity > 95%. ES-MS m/z 408 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ

10.61 (s, 1H), 7.63 (d, *J* = 2.45 Hz, 1H), 7.34 (dd, *J* = 2.73, 8.69 Hz, 1H), 7.00 (d, *J* = 8.85 Hz, 1H), 4.20 – 4.32 (m, 2H), 3.34 – 3.47 (m, 6H), 3.13 – 3.20 (m, 2H), 2.42 – 2.48 (m, 2H), 1.90 – 1.98 (m, 2H), 1.49 – 1.62 (m, 1H), 1.39 – 1.48 (m, 2H), 1.08 (t, *J* = 7.18 Hz, 3H), 0.90 (d, *J* = 6.59 Hz, 6H).

4.4. Supporting Information

Synthesis and characterization of all compounds and biological assay methods are included in the supporting information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

PM	plasmepsin
SPP	signal peptide peptidase
HIV	human immunodeficiency virus
SAR	structure-activity relationships
BACE	beta-site APP cleaving enzyme 1 or beta-secretase
TCAMS	Tres Cantos Antimalarial Set
RBC	red blood cells
CatD	cathepsin D
CatE	cathepsin E
MLM	mouse liver microsomes
RLM	rat liver microsomes
HLM	human liver microsomes
PK	pharmacokinetics

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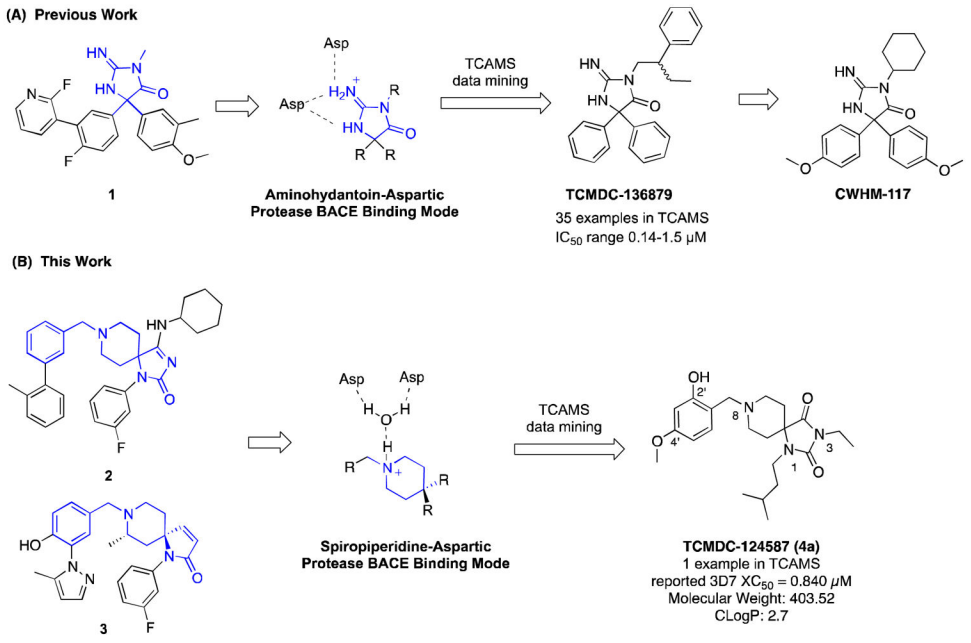


Figure 1.
Strategy to identify drug-like aspartic protease inhibitors as novel antimalarials.

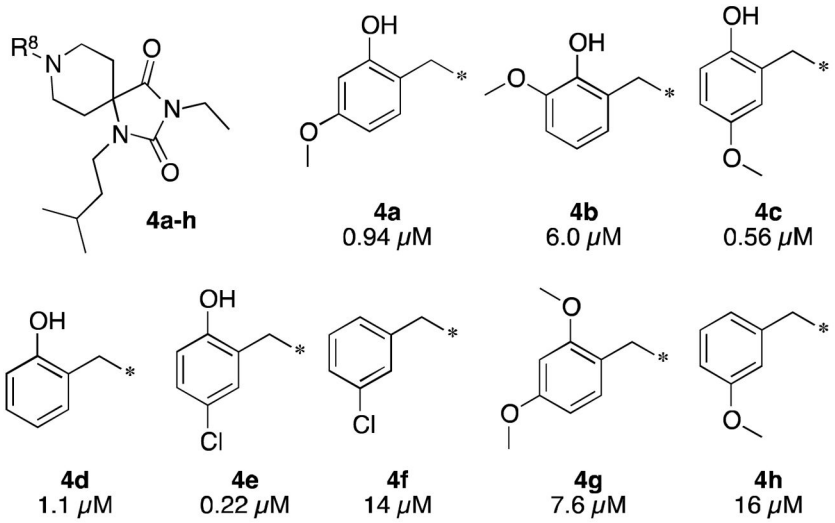


Figure 2.

Preliminary R^8 Structure-Activity Relationships. Reported potencies are IC_{50} values in *P. falciparum* 3D7 infected erythrocytes.

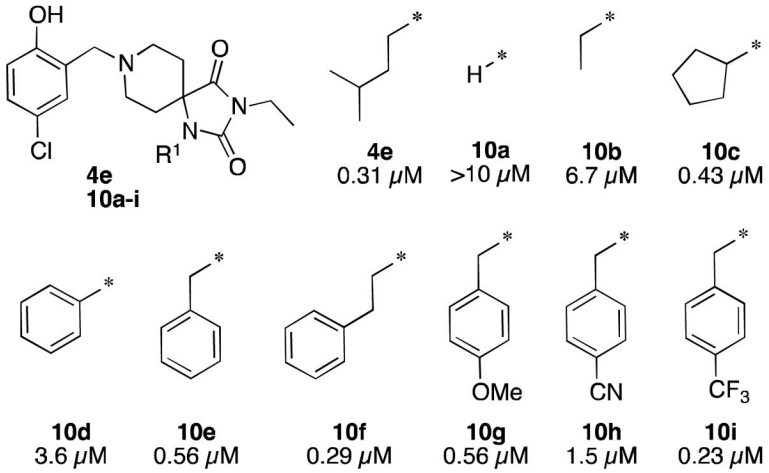


Figure 3. R^1 Structure-Activity Relationships

Reported potencies are IC_{50} values in *P. falciparum* 3D7 infected erythrocytes.

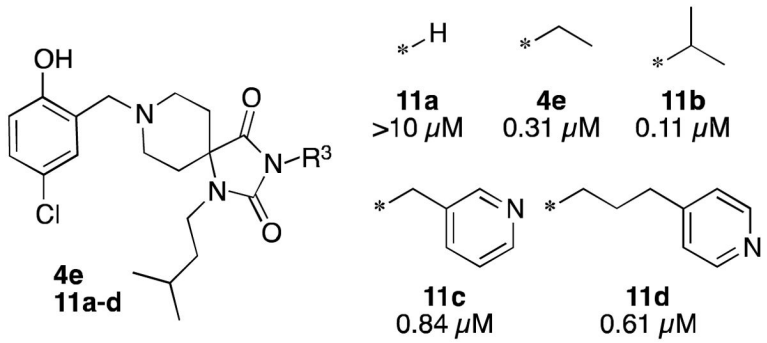


Figure 4. R^3 Structure-Activity Relationships

Reported potencies are IC_{50} values in *P. falciparum* 3D7 infected erythrocytes.

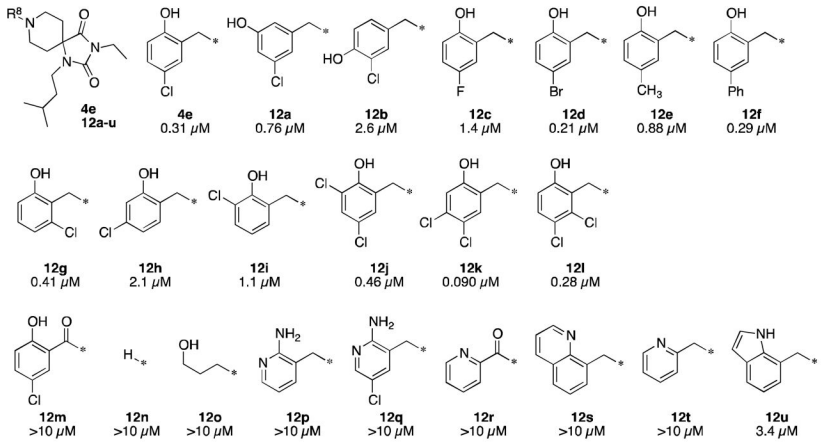
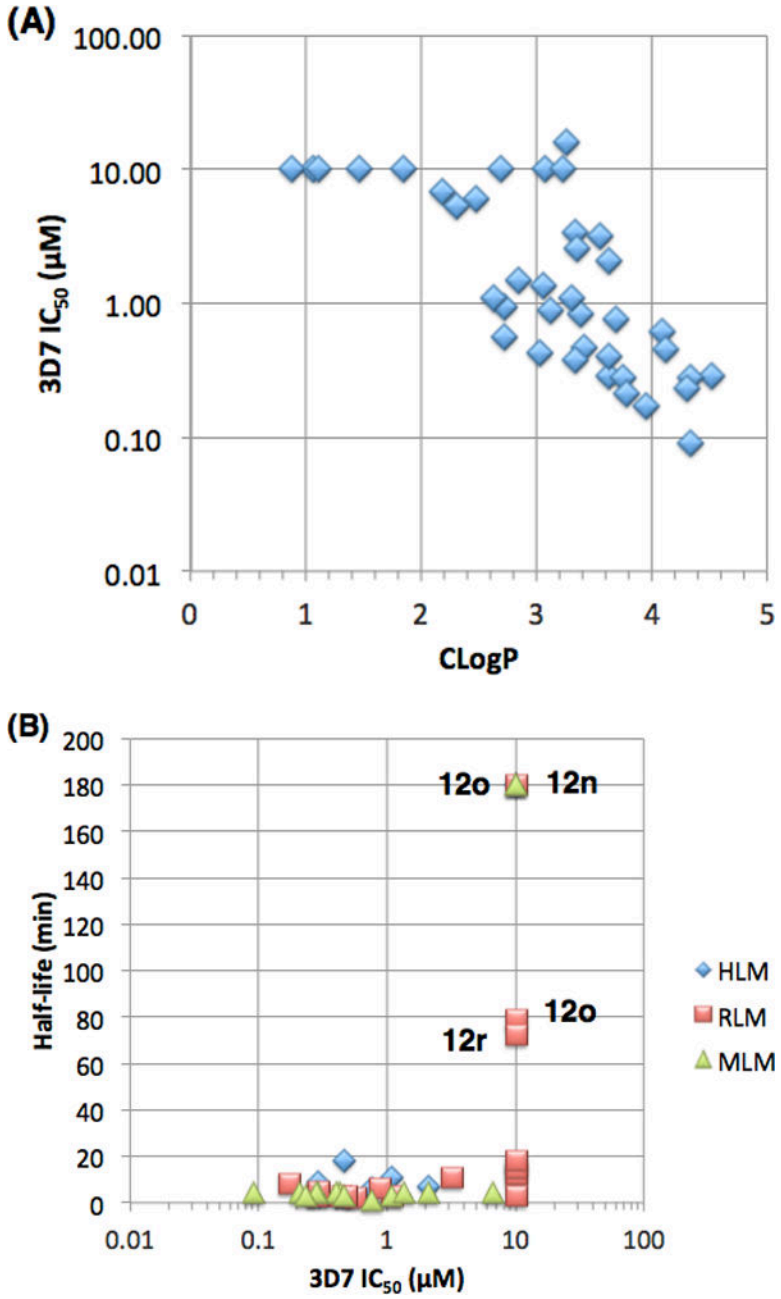


Figure 5. Preliminary R^8 Structure-Activity Relationships

Reported potencies are IC_{50} values in *P. falciparum* 3D7 infected erythrocytes.



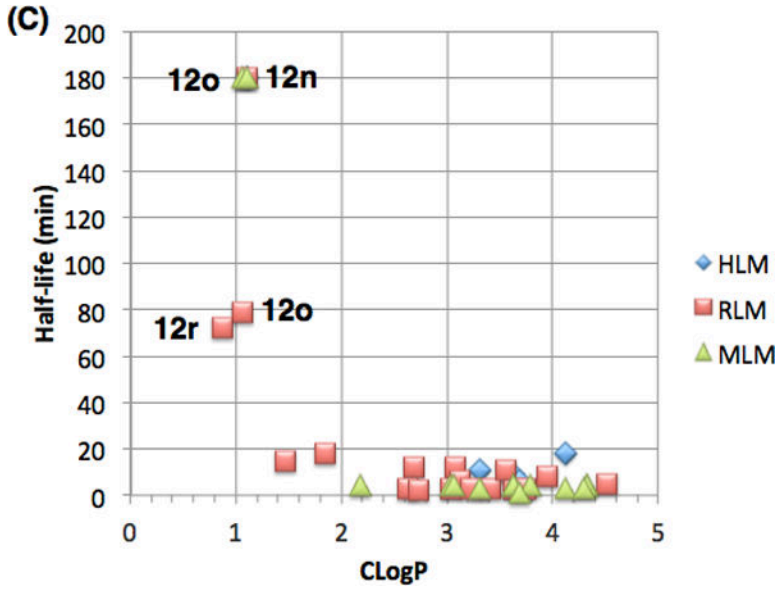
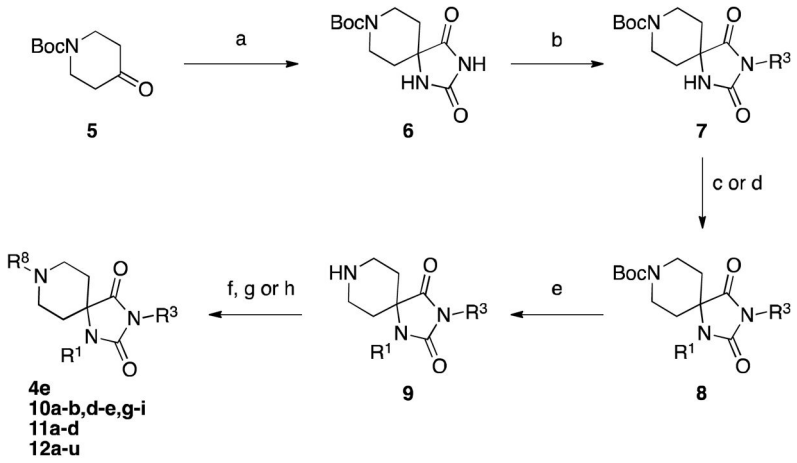


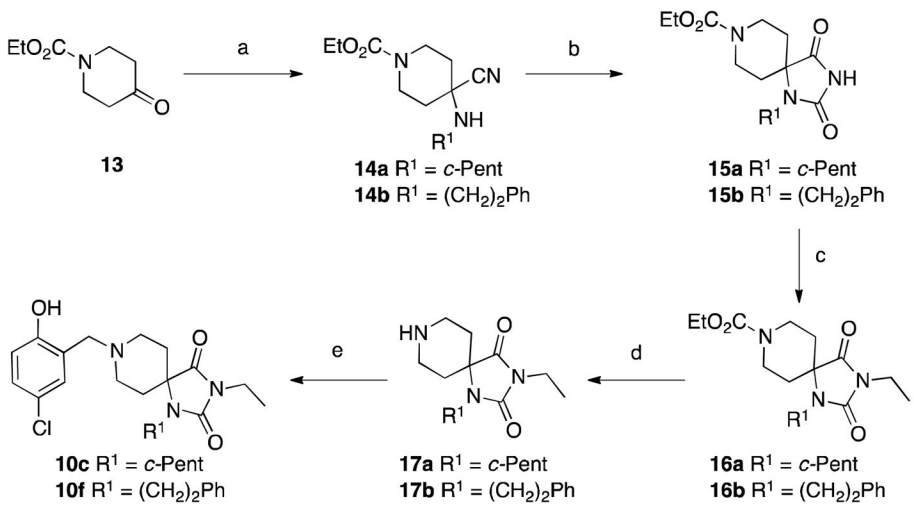
Figure 6. Relationship between 3D7 potency, metabolic stability and lipophilicity

(A) Plot of *Pf* 3D7 potency as a function of CLogP. (B) Plot of *Pf* 3D7 potency versus metabolic stability in mouse, rat and human liver microsomes (MLM, RLM and HLM). (C) Plot of metabolic stability in liver microsomes as a function of CLogP.



Scheme 1.

Reagents and Conditions: (a) KCN, $(NH_4)_2CO_3$, aq. MeOH; (b) R^3X, K_2CO_3, DMF; (c) NaH, R^1X, DMF; (d) ArI, CuI, 2,2,6,6-tetramethyl-3,5-heptanedione, Cs_2CO_3; (e) HCl or TFA; (f) RCHO, $Na(OAc)_3BH$, DMF; (g) RCO_2H, EDC, HOBt, NEM, DCM; (h) R^8Br, K_2CO_3, CH_3CN.



Scheme 2.

Reagents and Conditions: (a) R^1NH_2-HCl, KCN, aq. MeOH; (b) i. KOCN, AcOH, H_2O; ii. aq. HCl; (c) NaH, EtI, DMF; (d) KOH, EtOH; (e) RCHO, $Na(OAc)_3BH$, DMF