2-Oxotetrahydroquinoline-Based Antimalarials with High Potency and Metabolic Stability

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Abstract: We report a series of novel inhibitors of protein farnesyltransferase based on the 2-oxotetrahydroquinoline scaffold. We developed an efficient synthesis of these compounds. These compounds show selective inhibtion of the malaria versus human farnesyltransferase and inhibit the growth of the malaria parasite in the low nanomolar range. Some of the compounds are at least an order of magnitude more stable to metabolic degradation than the corresponding tetrahydroquinolines.

Efforts to discover and develop novel antimalarial agents are being intensified as a result of the widespread development of resistance to well established antimalarials and the availability of novel funding mechanisms including the Medicines for Malaria Venture.[1] There are about 300–500 million malaria cases each year, resulting in 1–3 million deaths, mostly in Africa.[2] In an effort to speed up the development of novel antimalarial agents, we have explored potential drug targets in the parasite for which considerable expertise is already available through efforts in the pharmaceutical industry to develop drugs for nontropical diseases. We have applied this "piggy-back" medicinal chemistry toward the development of inhibitors of the enzyme protein farnesyltransferase as antimalarial agents.[3–5] Protein farnesyltransferase from the *Plasmodium falciparum* malarial parasite (*Pf*-PFT[a]) appears to be an excellent drug target: (1) *Pf*-PFT inhibitors are cytotoxic rather than cytostatic to parasites.[6] (2) Inhibitors of human PFT are much less toxic to mammalian cells; in fact, they are being advanced through clinical trials for the treatment of leukemia in elderly patients who do not tolerate other chemotherapeutic regiments very well.[7] (3) Although it has not been possible to obtain recombinant *Pf*-PFT, partially purified enzyme from malaria-infected red blood cells is available for analysis of thousands of inhibitors.[8] (4) A structural homology model of the active site of *Pf*-PFT can be built using numerous X-ray structures of mammalian PFT with bound inhibitors.[9]

In previous work we discovered that tetrahydroquinoline (THQ)-based PFT inhibitors (Figure 1), first developed at Bristol-Myers Squibb as inhibitors of human PFT with anticancer potential,[10] are potent inhibitors of *Pf*-PFT and malarial growth. Our most potent compounds in the series include **29** (Figure 1) that inhibits *Pf*-PFT enzymatic activity in vitro with

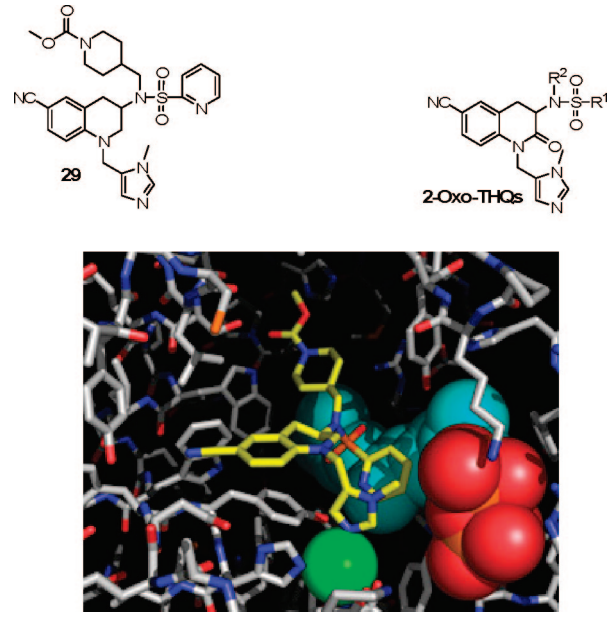

Figure 1. (Left) Chemical structure of **29**, a THQ-based *Pf*-PFT inhibitor. (Right) General structure of substituted 2-oxo-THQs. (Below) X-ray structure of **29** bound to mammalian PFT with the hydrophobic part of farnesyl diphosphate in cyan, the diphosphate part in red, and the Zn^{2+} in green (PDB coordinates available, 2r2l).

an IC_{50} (concentration that causes 50% enzyme inhibition) of 0.5 nM and arrests the growth of *P. falciparum* in human red cells with an ED_{50} (concentration that causes 50% inhibition of growth) of 15 nM.[6,12] The X-ray structure of **29** bound to mammalian PFT shows that the nonmethylated N of the imidazole ring directly binds to the active site Zn^{2+}. It also shows that the 6-CN on the tetrahydroquinoline ring, the group attached to the sulfonamide sulfur, and the group attached to the sulfonamide N pack into pockets on the enzyme (Figure 1).[9]

Values of ED_{50} in the low nanomolar region are thought to be adequate for antimalarial drugs. Therefore, compounds such as **29** have acceptable potency. The problem that we encountered with THQs in general is their high rate of metabolic degradation by one or more cytochrome P450s present in liver microsomes.[11] This translated to a rapid rate of clearance of THQs from the serum of mice and rats that were dosed orally.[11] Detailed studies using combined liquid chromatography/mass spectrometry together with isotopically labeled THQs led to the realization that the major route of metabolic degradation, at least for some of the compounds, is loss of the imidazole-containing side chain attached to N-1 of the THQ ring (Figure 2). This cytochrome P450-catalyzed reaction can occur by one of two pathways (Figure 2).

Pathway 1 involves initial electron transfer from N-1 to the enzyme to give a radical cation, which undergoes further hydrogen atom transfer to the P450 to give the iminium ion, which spontaneously breaks down to the amine plus aldehyde. Pathway 2 involves initial hydrogen atom transfer from the methylene to the enzyme to form a C-centered radical followed by hydroxyl rebound to give the carbinolamine, which spontaneously decomposes to the observed metabolites, amine plus aldehyde. We reasoned that placement of a 2-oxo group onto the THQ ring (2-oxo-THQ, Figure 1) would suppress this N-dealkylation reaction regardless of which pathway was

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[a] Abbreviations: *Pf*-PFT, protein farnesyltransferase from *P. falciparum*; THQ, tetrahydroquinoline.

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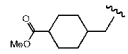
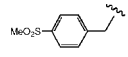
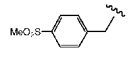
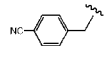
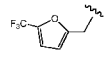
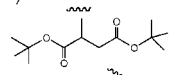
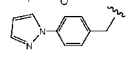
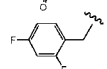
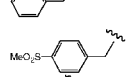
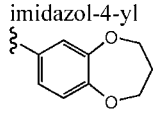
Table 1. *Pf*-PFT inhibition and Antimalarial Activity of 2-Oxo-THQs[a]

			Pf-PFT % inhibition at indicated concentration			ED_{50} (nM)	
Compd.	R_1	R_2	50 nM	5 nM	0.5 nM	3D7	K1
5(bromo)	1-Me-imidazol-4-yl	H	60	15	5	5000	3000
6(bromo)	2-pyridyl	H	36	15	0	5000	5000
7	1-Me-imidazol-4-yl	H	79	41	0	ND	ND
8	1-Me-imidazol-4-yl		98	83	21	105	ND
9	1-Me-imidazol-4-yl		99	91	23	80	ND
10(bromo)	1-Me-imidazol-4-yl		86	26	0	850	ND
11	1-Me-imidazol-4-yl		98	86	25	200	100
12(bromo)	1-Me-imidazol-4-yl		98	81	24	105	ND
13	1-Me-imidazol-4-yl		86	41	4	3200	2000
14	1-Me-imidazol-4-yl		98	88	45	175	100
15	1-Me-imidazol-4-yl		91	56	26	2100	2100
16	1-Me-imidazol-4-yl		92	67	1	625	325
17	1-Me-imidazol-4-yl		100	97	62	35	30
18	1-Me-imidazol-4-yl		0	0	0	5000	5000
19	1-Me-imidazol-4-yl		100	97	50	80	150
20	1-Me-imidazol-4-yl		98	79	26	ND	ND
21	1-Me-imidazol-4-yl		80	27	4	ND	ND
22	1-Me-imidazol-4-yl		45	12	9	5000	5000
23	1-Me-imidazol-4-yl		97	71	33	800	ND
24	1-Me-imidazol-4-yl		8	5	0	5000	ND
25	2-pyridyl		91	60	15	1700	275
26	1-Me-5-Me-imidazol-4-yl		24	11	6	5000	5000
27			22	4	0	5000	ND
28	3-F-phenyl		56	19	0	3600	ND

[a] See Figure 1 for the generic structure of 2-oxo-THQs. In the table, "ND" indicates not determined and "(bromo)" indicates that the compound has Br instead of CN at position 6 of the 2-oxo-THQ ring.

operative. In the context of pathway 1, the addition of a 2-oxo group leads to a lactam in which the lone pair on N-1 is less susceptible to oxidation by cytochrome P450 owing to the amide resonance. If pathway 2 applies, the lone pair on N-1 is less able to donate into the electron-deficient p-orbital of the C-centered radical.

A highly efficient route to 2-oxo-THQs, novel structures not reported previously, is summarized in Scheme 1 (full experimental details given in Supporting Information).

Commercially available aniline **1** undergoes reductive amination with commercially available 5-formyl-1-methylimidazole. Conversion of the methyl ester to the primary chloride **2** is carried out by a standard sequence. Malonic ester synthesis gives lactam **3**, which is converted to lactam **4** by acid catalyzed hydrolysis and decarboxylation. The 3-amino group is sulfonated and alkylated, and the 6-bromo is converted to 6-CN via Pd-catalyzed exchange to afford the 2-oxo-THQs. Several 2-oxo-THQs were made by this route (Supporting Information) and are shown in Table 1.

We prepared and tested 25 2-oxo-THQs for their ability to inhibit *Pf*-PFT in vitro using 3 inhibitor concentrations (0.5, 5, and 50 nM) (Table 1). We also tested the compounds to

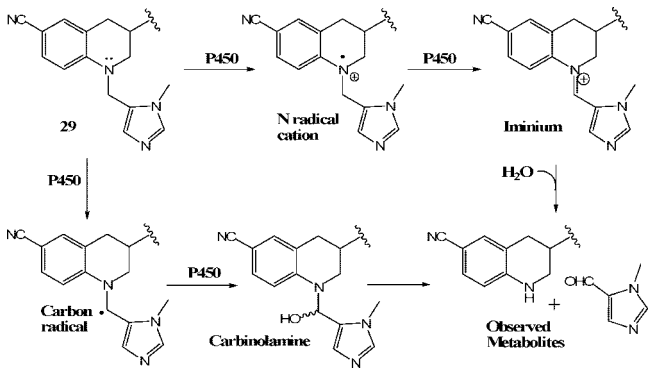
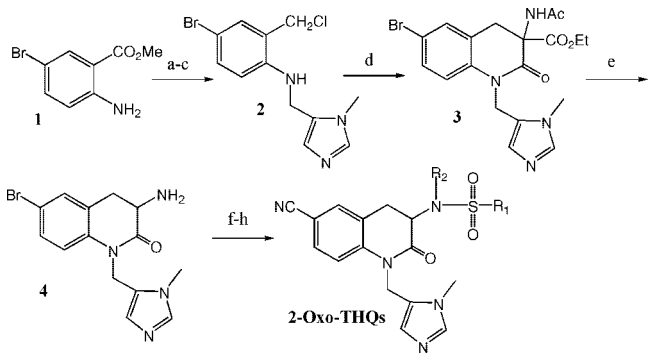


Figure 2. Two pathways for cytochrome P450-catalyzed loss of the substituent from N-1 of the THQ. Pathway 1 (top route) involves initial transfer of a N-1 lone pair electron, whereas pathway 2 (bottom route) proceeds via a C-centered radical.

Scheme 1[a]



[a] Reagents and conditions: (a) 5-formyl-1-methylimidazole, CF_3CO_2H, EDC, Et_3SiH, 50 °C, 48 h (40%); (b) $LiAlH_4$, THF, 0 °C to room temp, 2 h (72%); (c) $SOCl_2$, $CHCl_3$, reflux, 2 h (90%); (d) diethyl acetamidomalonate, NaH, DMF, 120 °C, 12 h (65%); (e) concentrated HCl, reflux, 12 h, (75%); (f) R_1SO_2Cl, DIPEA, CH_3CN, room temp, 12 h (65%); (g) R_2Br, Cs_2CO_3, DMF, room temp, 12 h (55%); (h) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, microwave 2.5 min (78%).

determine the ED_{50} for the arrest of growth of two strains of *P. falciparum* parasites (chloroquine sensitive 3D7 and chloroquine resistant K1 strains) in human red blood cells (Table 1). In general there is a good correlation between potency of inhibition of *Pf*-PFT in vitro and parasite growth arrest. The importance of the 6-CN group is apparent from data obtained with two compounds that have a 6-Br group (**5** and **6**). Among the R_1 groups examined, the *N*-methylimidazole-4-yl gives the most potent compounds. The best compound in the series in terms of antimalarial potency is **17** with an exceptional ED_{50} of 30 nM. A few other compounds were discovered to have ED_{50} below 100 nM.

Next we examined the metabolic stability of our most potent compounds by measuring the half-life for compound loss upon incubation with mouse liver microsomes in the presence of an NADPH regeneration system. Results are summarized in Figure 3 and Table 2 along with the half-life for the corresponding THQs without the 2-oxo group (**30**−**34**). Some of the 2-oxo-THQs show exceptional stability to microsomal degradation; for example, **9** and **11** are ∼10-fold more stable than the corresponding THQ analogues lacking the 2-oxo group (Figure 3, Table 2).

Despite testing more than 100 THQs for metabolic stability, we have not been able to find compounds lacking the 2-oxo groups that displayed half-times greater than 30 min.[11] Other 2-oxo-THQs in Table 2 are still destroyed by mouse liver microsomes within shorter times presumably because of routes other than loss of the Zn^{2+}-binding *N*-methylimidazole arm, but this was not investigated.

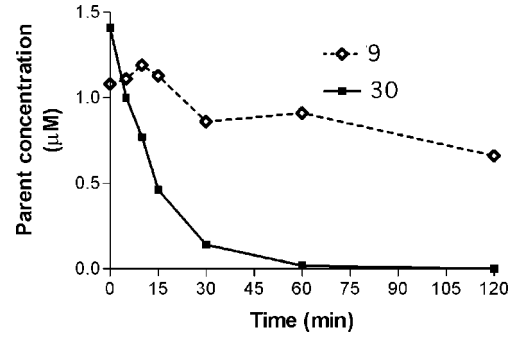


Figure 3. In vitro metabolism by mouse liver microsomes of the 2-oxo-THQ **9** (half-time of 133 min) and the structurally matched THQ lacking the 2-oxo group (**30**) (half-time of 16 min).

Table 2. Half-Times (min) for Metabolic Degradation of 2-Oxo-THQs and THQs by Mouse Liver Microsomes

2-oxo-THQs	half-time[a]	THQ[b]	half-time[a]
9	>120, 133	**30**	16, 10
11	>60, 144	**31**	17
15	40	**33**	3
17	21	**34**	10, 7
19	9		
28	31		

[a] Where two numbers are listed, this is the half-time values for two independent experiments. [b] Data for THQs is from ref 11. The structure of the THQ is same as that of 2-oxo-THQ but lacking the 2-oxo group.

Table 3. Inhibition of Rat PFT

	rat PFT, % inhibition at indicated concentration			
compd	5000 nM	500 nM	50 nM	5 nM
9	73	23	0	0
10 (bromo)	56	0	0	0
11	86	62	0	0
14	61	5	0	0
17	92	75	23	0
19	84	54	4	0

We also tested some of the 2-oxo-THQs for their ability to inhibit human mammalian (rat) PFT using an in vitro assay described previously,[6] and results are shown in Table 3. It can be seen that the 2-oxo-THQs are much less potent on rat PFT compared to *Pf*-PFT.

In summary, a new series of inhibitors of the malarial protein farnesyltransferase (*Pf*-PFT), 2-oxo-THQs, have been designed on the basis of consideration of the structure of the enzyme–inhibitor complex and the knowledge of the pathway of liver metabolism. Of the 25 compounds prepared, a subset showed sufficient potency and metabolic stability to warrant further development of 2-oxo-THQs as novel antimalarial agents. As this series is expanded, we will focus on continued improvement in potency against parasite growth while maximizing oral bioavailability. An efficient route for the synthesis of 2-oxo-THQs was developed, which is important for reducing the cost of goods, an important requirement in antimalarial drug discovery that is often underestimated.

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Supporting Information Available: Experimental details including the synthesis of all compounds and assay procedures and HPLC traces of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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