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2-Oxo-tetrahydro-1,8-naphthyridines as selective inhibitors of malarial protein farnesyltransferase and as anti-malarials

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Abstract—A new class of 2-oxo-tetrahydro-1,8-naphthyridine-based protein farnesyltransferase inhibitors were synthesized and found to inhibit protein farnesyltransferase from the malaria parasite with potencies in the low nanomolar range. The compounds were much less potent on mammalian protein prenyltransferases. Two of the compounds block the growth of malaria in culture with potencies in the sub-micromolar range. Some of the compounds were found to be much more metabolically stable than previously described tetrahydroquinoline-based protein farnesyltransferase inhibitors.

Malaria remains one of the key parasitic diseases present today with more than 400 million acute illnesses and at least 2–3 million deaths annually throughout the tropical and subtropical regions of the world. The malaria parasite is able to become resistant to chemotherapeutic agents. For example, in many places there is a major problem of resistance to chloroquine, a 4-aminoquinoline that has been the mainstay of treatment for malaria for many years. Hence, there is an urgent need for the development of new drugs against new molecular targets.[1]

Protein prenylation has been vigorously studied over the past ~15 years because it is found on several signaling proteins (including heterotrimeric G proteins) that connect cell surface receptors to intracellular effectors, and also on Ras proteins, one of the most common oncoproteins found in human tumors. Protein farnesyltransferase (PFT) transfers the farnesyl group from farnesyl diphosphate to the SH of the cysteine near the C-terminus of proteins such as Ras. PFT inhibitors (PFTIs) have been extensively developed as anti-cancer agents because of their ability to block tumor growth in experimental animals. We have been pursuing PFTIs as anti-malarial and anti-trypanosome agents because these compounds are much more toxic to these parasites than to mammalian cells, and there are a large number of lead compounds from which to launch an anti-parasite drug discovery program (piggy-back medicinal chemistry).[2–4] We have developed tetrahydroquinoline (THQ)-based PFTIs as anti-malarials with low nanomolar potency on parasite PFT and on parasites cultured in vitro.[3–7] One of our lead compounds, **1** (Fig. 1), kills *Plasmodium falciparum* growth in vitro with an ED_{50} of 16 nM, shows high oral bioavailability, and is able to cure rats infected with rodent malaria.[6]

Unfortunately, high doses of **1**, 50 mg/kg, were required for cures in rodents because of rapid compound clearance. In vitro microsome metabolism studies suggest that the major culprit is cytochrome P450-catalyzed loss of the imidazole-containing side chain (which binds to the active site Zn^{2+} of PFT) leading to **2** (Fig. 1). This reaction starts either with P450-catalyzed hydrogen atom abstraction from the CH_2 group attached to N1 of the tetrahydroquinoline ring to give a C-centered radical or enzyme-catalyzed oxidation of N1 to give

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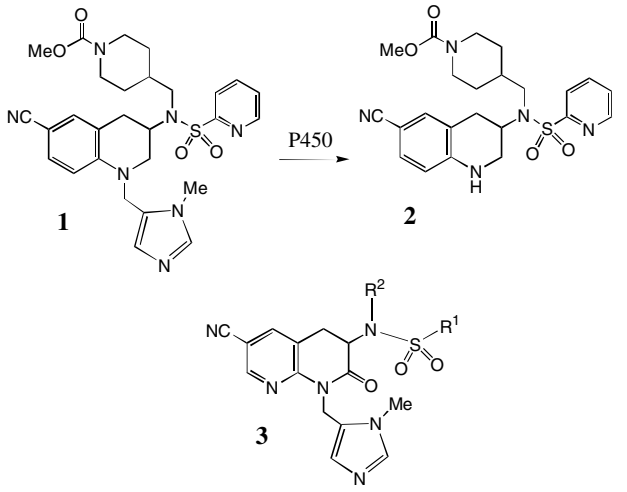
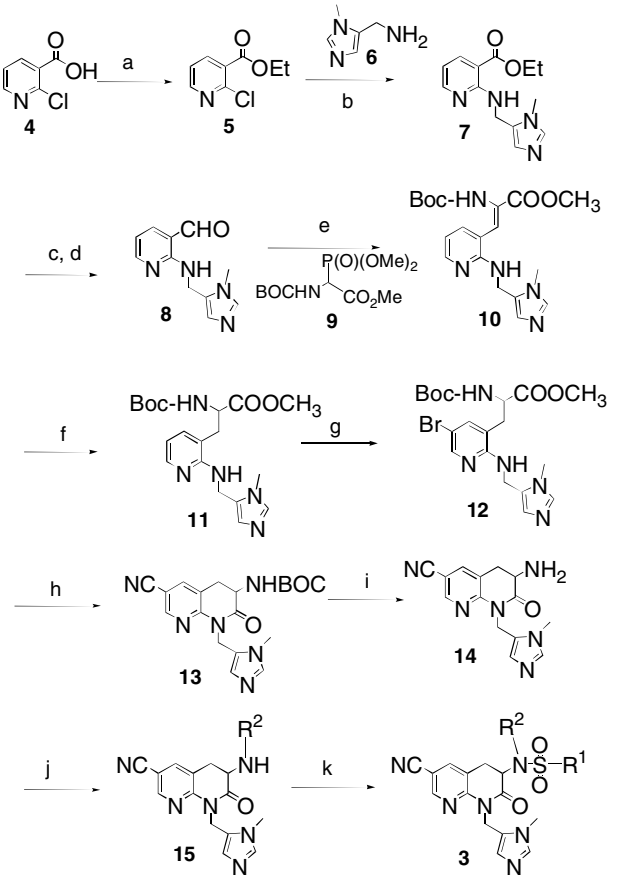


Figure 1. Compound **1** is a tetrahydroquinoline-based PFTI that is metabolized by cytochrome P450 to give compound **2**. Compound **3** shows the general structure of the 2-oxo-tetrahydro-1,8-naphthyridine-based PFTIs prepared in the current study.

the N-centered radical cation. Regardless of the mechanism, we envisioned that placement of an oxo group at the 2-position of the tetrahydroquinoline ring and a N in place of C-8 would reduce P450-catalyzed radical formation due to a rise in the oxidation potential of the N1 lone pair electrons (due to involvement of the lone pair in resonance with the carbonyl and the pyridine N). Thus, we set out to prepare 2-oxo-tetrahydro-1,8-naphthyridine-based PFTIs exemplified by **3** (Fig. 1). Considering the X-ray structure of tetrahydroquinoline PFTIs bound to mammalian PFT and a homology model of the active site of malarial PFT,[4,6] it appears that addition of the 2-oxo and 8-aza groups to the tetrahydroquinoline scaffold would be tolerated.

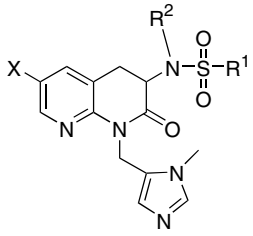
Compounds were prepared following the synthetic sequence illustrated in Scheme 1. Ethyl chloronicotinate **5** was prepared from 2-chloro nicotinic acid **4**, and installation of the imidazole was accomplished by nucleophilic substitution conditions to give **7**. This was followed by reduction of the ester group and subsequent oxidation to yield **8**. The Wittig olefination of compound **8** with Boc protected phosphonoacetate **9** gave **10** followed by catalytic hydrogenation over palladium in methanol to give **11**. Subsequent bromination with Br_2 in acetic acid afforded 6-bromo analogue **12**, which was converted to the corresponding 6-cyano derivative **13** by treatment with zinc cyanide and tetrakis(triphenylphosphine)palladium in dimethylformamide. Removal of Boc group with trifluoroacetic acid in dichloromethane afforded the key intermediate 3-amino-6-cyano-2-oxo-tetrahydro-1,8-naphthyridine **14**. Completion of target molecules was accomplished following a 2-step sequence of reductive amination and sulfonamide formation. If sulfonation was carried out first followed by alkylation of the sulfonamide N with R^2Br, the observed product was the enamine with a double bond in the 3,4-position of the lactam ring (due to elimination of the sulfinate). Full synthetic details are available as Supplementary Data.

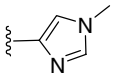
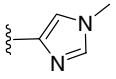
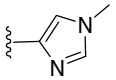
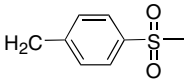
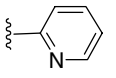
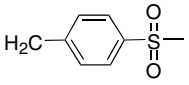
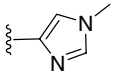
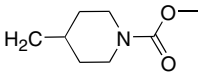
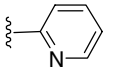
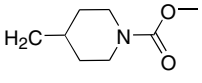


Scheme 1. Reagents: (a) $SOCl_2$, EtOH, 80%; (b) Et_3N, DMF, 60%; (c) $LiAlH_4$, THF, 70%; (d) MnO_2, CH_2Cl_2, 75%; (e) tetramethylguanidine, CH_2Cl_2, 65%; (f) H_2/Pd-C, CH_3OH, 50%; (g) Br_2, CH_3COOH, 65%; (h) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF 35%; (i) 20% CF_3COOH, CH_2Cl_2, 100%; (j) R^2-CHO, $NaCNBH_3$, CH_3OH, 55–60%; (k) R^1-SO_2Cl, DIPEA, CH_2Cl_2, 15–20%.

Our previous structure–activity data on THQ-based inhibitors of malarial PFT led to the discovery of compounds with R^1 = *N*-methyl-4-imidazolyl or 2-pyridyl (i.e., **1**) as being potent inhibitors of malarial PFT.[7] In Table 1, we report anti-malarial results obtained with 2-oxo-tetrahydro-1,8-naphthyridine-based PFTIs with R^1 = *N*-methyl-4-imidazolyl or 2-pyridyl and with variation of the R^2 group. Compounds with R^1 = *N*-methyl-4-imidazolyl conferred the best in vitro activity against *Plasmodium falciparum* PFT (**18** and **20** showed 98% and 95% inhibition at 50 nM, respectively) compared to compounds with R^1 = 2-pyridyl (**21** and **19** showed 88% and 48% inhibition at 50 nM, respectively). We also tested the compounds for their ability to block the growth of *Plasmodium falciparum* in human red blood cell cultures. Values of ED_{50}, the concentration of compound that reduces parasite growth by 50%, are listed in Table 1. Two malarial strains were studied, 3D7, which is chloroquine sensitive, and K1, which is chloroquine resistant. Compounds **18**, **20**, and **21** showed good potency, with values of ED_{50} in the 175–420 nM range (Table 1). These compounds are also the most potent in the series studied in inhibiting *Plasmodium falciparum* PFT in vitro. Testing on malarial PFT and on parasites was carried out as described.[6]

Table 1. Structure, PFT inhibition, anti-malarial activity, and microsomal metabolism of 2-oxo-tetrahydro-1,8-naphthyridine-based PFTIs



Compound	X	R^1	R^2	Malaria PFT enzyme % inhibition at the indicated inhibitor concentration (nM)				ED_{50} for inhibition of parasite growth in vitro[a] (nM)		Microsome metabolism half-time[b] (min)	
				500	50	5	0.5	3D7	K1	Naph	THQ
16	Br		H	74	28	0	0	>5000	>5000	ND	ND
17	CN		H	94	66	32	15	>5000	>5000	ND	ND
18	CN			99	98	80	27	420	300	52	15.6
19	CN			91	48	3	0	3100	>5000	15	ND
20	CN			98	95	71	16	350	175	>120	5.4
21	CN			97	88	33	0	320	310	ND	3.8

[a] ED_{50} is the concentration of compound that inhibits the growth of parasites by 50% (chloroquine sensitive strain 3D7 or chloroquine resistant strain K1) in red blood cell cultures (measured according to Ref. 6).

[b] Given is the half-time for loss of parent compound when incubated with mouse liver microsomes according to the procedure given in Ref. 6. Compounds tested are: (1) column labeled Naph, the 2-oxo-tetrahydro-1,8-naphthyridines shown in the table; (2) column labeled THQ, the corresponding tetrahydroquinoline-based PFT (analogs of **1**) with the same R^1 and R^2 groups as the indicated 2-oxo-tetrahydro-1,8-naphthyridine. ND, not determined.

Next, we tested the stability of the 2-oxo-tetrahydro-1,8-naphthyridine-based inhibitors toward metabolism by mouse liver microsomes in vitro. For comparison, in Table 1 we also include previous data for in vitro metabolism of the corresponding tetrahydroquinolines. Compound **19** was found to be about 3-folds more stable than the corresponding tetrahydroquinoline. For compound **20**, the increase in stability is dramatic, >20-fold. In vitro microsome metabolism studies were carried out as described.[6] All compounds in Table 1 were found to be stable after incubation in aqueous buffer at pH 2 for 24 h.

We tested four of the compounds for inhibition of mammalian protein prenyltransferases, and results are shown in Table 2. It can be seen that the compounds are reasonably selective for the malarial versus rat PFT. For example, compounds **17**, **18**, and **20** at 5 nM inhibits malarial PFT by 32%, 80%, and 71%, but inhibit rat PFT at this concentration only by 0%, 0%, and 7%, respectively. All four compounds are very poor inhibitors of rat PGGT-I.

Table 2. Inhibition of rat PFT and protein geranylgeranyltransferase-I (PGGT-I) by 2-oxo-tetrahydro-1,8-naphthyridine-based PFTIs

Compound	Rat PFT, % inhibition at the indicated inhibitor concentration[a] (nM)					Rat PGGT-I, % inhibition at 1 μM inhibitor[b]
	5000	500	50	5	0.5	
16	51	8	8	5	0	16
17	39	0	1	0	0	0
18	47	9	0	0	0	0
20	85	71	28	7	0	5

[a] PFT assays were carried out as in Ref. 7.

[b] PGGT-I assays were carried out as for PFT assays except rat PGGT-I (5 ng), 5 μM H-Ras-CVLL, and 0.65 μM [^{3}H]geranylgeranyl pyrophosphate were used. [8]

In conclusion, we have developed a new class of PFT inhibitors based on the 2-oxo-tetrahydro-1,8-naphthyridine scaffold that are more potent on malaria PFT than on the mammalian enzyme. These compounds are metabolically more stable than the tetrahydroquinoline lead compounds. The route of synthesis of these novel compounds was also developed. The most potent compounds in the series inhibit the malarial PFT with potencies in the low nanomolar range and kill cultured parasites in the hundreds of nanomolar range. Future studies will focus on the oral bioavailability and antimalaria efficacy of these compounds in rodents.

Acknowledgments

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Supplementary data

Experimental details for the synthesis of representative compounds are available as Supplementary data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.104.

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