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Peptidomimetic Inhibitors of Protein Farnesyltransferase Show Potent Antimalarial Activity

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Abstract—Malaria continues to represent a very serious health problem in the tropics. The current methods of clinical treatment are showing deficiencies due to the increased incidence of resistance in the parasite. In the present paper we report the design, synthesis, and evaluation of potential antimalarial agents against a novel target, protein farnesyltransferase. We show that the most potent compounds are active against *Plasmodium falciparum* in vitro at submicromolar concentrations. © 2001 Elsevier Science Ltd. All rights reserved.

Malaria is one of the most widespread infectious diseases in the tropics. Nearly 300 million new cases occur each year, resulting in more than one million deaths annually. The fatal cases are generally caused by the most virulent human malaria parasite, *Plasmodium falciparum*. Current clinical treatment involves the use of inexpensive antimalarial drugs such as chloroquine. However resistance has rendered many of these drugs ineffective. Other malaria control efforts have focused on mosquito eradication programs, the development of a vaccine against the parasites, and new drug targets such as protease inhibitors or isoprenoid biosynthetic pathway inhibitors.[1] However, the slow development of a vaccine and the pressing problem of chloroquine resistance have created an urgent need for identifying new drug targets in *Plasmodium falciparum* and for the development of effective new antimalarial agents. In this paper we report that a family of inhibitors of the enzyme protein farnesyltransferase (FTase) shows potent antimalarial activity in whole cell assays.

Mammalian FTase is a heterodimeric zinc metalloenzyme composed of a 48 kDa α-subunit and a 46 kDa β-subunit that catalyzes the transfer of a farnesyl group (C_{15}) from farnesyl pyrophosphate (FPP) to the thiol of a Cys residue near the carboxyl terminus of target proteins. The key C-terminal sequence that is recognized by FTase is CaaX, where C is cysteine, aa is an aliphatic dipeptide, X is often methionine, glutamine or serine. In contrast, the related prenyltransferase, protein geranylgeranyltransferase-I (GGTase-I), recognizes and geranylgeranylates (C_{20}) proteins that terminate in CaaX, where X is leucine or phenylalanine. In recent years, FTase has been a major target in the search for novel anticancer agents. This effort was initially based on the observation that the Ras family of G-proteins normally cycle between their GTP (active) and GDP (inactive) bound forms, to regulate intracellular signal transduction. However, in the mutated form of Ras that is found in over 30% of human cancers, the protein is locked in the GTP bound state, and this results in

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uncontrolled cell growth. The requirement that Ras be farnesylated and located in the plasma membrane for oncogenesis prompted many investigators to target FTase. In particular, we and others have reported peptidomimetics of the CaaX sequence[2–7] that are subnanomolar inhibitors of FTase and suppress tumor growth in nude mice without apparent gross toxicity.

Protein prenylation occurs in protozoan parasites. We have shown that protein farnesylation occurs in the trypanosomatids *Trypanosoma brucei, Trypanosoma cruzi,* and *Leishmania mexicana amazonensis*, and have purified, cloned, and expressed FTase from *T. brucei*.[8,9] Recently, the genes coding for the subunits of *P. falciparum* FTase have been identified in genome databases, and Chakrabarti et al. reported that an FTase inhibitor showed inhibition activity against the growth of *P. falciparum* in human red blood cells ($IC_{50} = 43$ μM).[10] These findings suggested that protein farnesylation in *P. falciparum* could be a new target for antimalarial agents. In this paper we report that a new series of tetrapeptide CaaX mimetics show significant inhibition against *P. falciparum* in cell culture.

Our strategy for the design of potent FTase inhibitors involved designing mimics of the CaaX tetrapeptide in which the central aliphatic dipeptide is replaced by a hydrophobic and rigid spacer, such as a substituted 4-aminobenzoyl group. This led to a family of highly potent inhibitors of FTase, such as FTI-276 (**1**), which has an IC_{50} against the mammalian enzyme of 0.5 nM, blocked protein farnesylation in mammalian cells and slowed the growth of human tumors in nude mouse models.[7] The discovery of FTase in parasites and the likely critical role of farnesylated G-proteins in the parasitic life cycle prompted us to investigate the effect of our peptidomimetics as antiparasitic agents. We recently showed that FTI-276 is also an effective inhibitor of *T. brucei* FTase ($IC_{50} = 1.7$ nM) and blocks parasite growth in the 10–50 nM range.[8] On the basis of these findings with trypanosomatids we decided to investigate the potential application of CaaX peptidomimetics against *P. falciparum*. Modifications to the structure of the peptidomimetics were based on the recent crystal structure of mammalian FTase bound to a CaaX substrate and an unreactive farnesyl diphosphate analogue. This showed that the tetrapeptide binds in an extended conformation with the Cys thiol coordinating to a zinc in the active site.[11] In order to overcome the problem of metabolic instablility of the thiol group in a parasitic cell growth experiment, we replaced the cysteine residue in FTI-276 with imidazole as an alternative metal binding functional group.

Furthermore, an imidazole group readily allows the introduction of various substituents to probe the presence of hydrophobic and/or polar domains near the active site. The compounds investigated in this study are listed in Chart 1. The basic synthetic approach used for the preparation of the CaaX peptidomimetics is shown in Scheme 1 for the synthesis of **2**–**11**. Suzuki aryl coupling of 2-bromo-4-nitrotoluene with phenylboronic acid followed by oxidation of the methyl group afforded the basic biphenyl scaffold, 2-phenyl-4-nitrobenzoic acid. For compounds **2**–**6**, the scaffold was coupled with methionine methyl ester, and was reduced to the aniline derivative. Reductive amination with the corresponding 1-substituted 4- or 5-formylimidazole followed by hydrolysis gave the free carboxylic acids. For compounds **7**–**11**, the scaffold was converted to the *t*-butyl ester, and was hydrogenated to the amine. After reductive amination with 1-(4-cyano)benzyl-5-formylimidazole[2] under treatment with $TiCl_4$, followed by deprotection under acidic conditions, the resulting benzoic acid derivative was coupled with an alkylamine using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI)/1-hydroxybenzotriazole (HOBt) to give the corresponding amides.

The whole cell inhibition assay of *P. falciparum* growth in human red blood cells was carried out in vitro in a 48 h [^{3}H]-hypoxanthine incorporation assay.[12] The ability of the peptidomimetic FTase inhibitors to slow the growth of *P. falciparum* in human red blood cells is reported in Table 1 as ED_{50} values and % inhibition at 20 μM. These two experiments were carried out independently on the same strain (3D7). Comparison is also made in Table 1 to the IC_{50} values for inhibition of mammalian FTase and of H-Ras processing in NIH 3T3 cells.

Compound **1** (FTI-276) did not show any apparent inhibition of parasite growth, whereas all of the imidazole-containing derivatives (with the exception of **4** and **12**) inhibited parasite growth at concentrations below 30

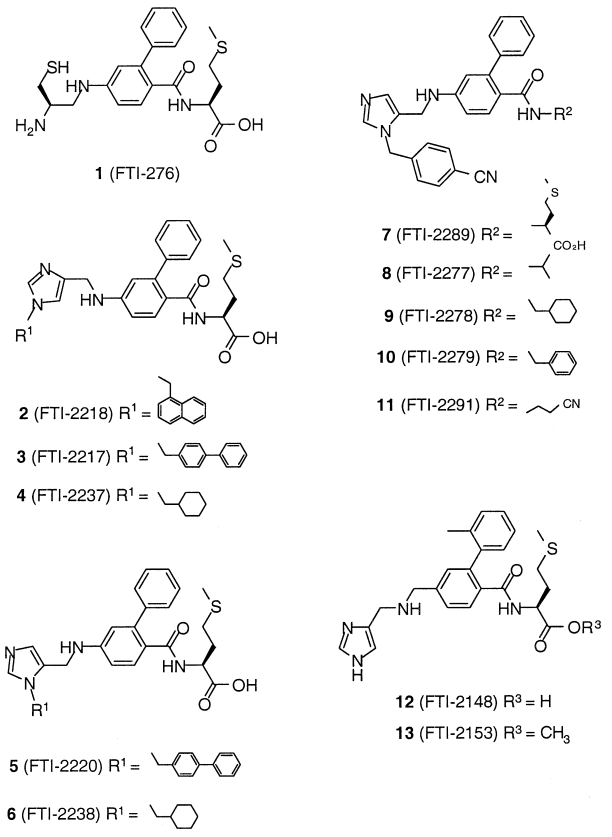
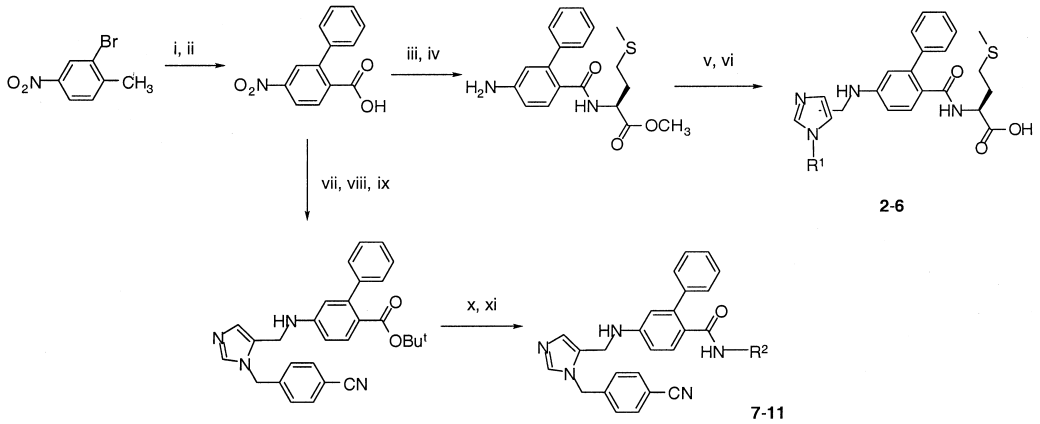


Chart 1.



Scheme 1. (i) $PhB(OH)_2$, $Pd(OAc)_2$, K_2CO_3, 89%; (ii) $KMnO_4$, 100%; (iii) HCl·H-Met-OMe, EDCI, HOBt, NEt_3, 89%; (iv) $SnCl_2$, AcOEt, 90%; (v) 1-substituted 4- or 5-formylimidazole, $NaBCNH_3$, 54–91%; (vi) LiOH, THF, 72–92%; (vii) $SOCl_2$, Bu^tOK, 72%; (viii) H_2/10% Pd–C, THF, 100%; (ix) 1-(4-cyano)benzyl-5-formylimidazole, $TiCl_4$, $NaBCNH_3$, 73%; (x) 50% TFA in CH_2Cl_2, 100%; (xi) R^2NH_2, EDCI, HOBt, NEt_3, 77–86%. (xii, for **7**) LiOH, THF, 90%.

Table 1. ED_{50} values for *P. falciparum* 3D7 growth inhibition and IC_{50} values for inhibition of mammalian FTase and of H-Ras processing

Compds	*P. falciparum* infected RBC ED_{50} (μg/mL)	*P. falciparum* infected RBC (%) inhibition[a]	Mammalian H-Ras processing IC_{50}, μM (IC_{50} for FTase, μM)
1 (FTI-276)	>30	nd	1 (0.0005)
2 (FTI-2218)	10	19.4	>100 (3)
3 (FTI-2217)	12	nd	>100 (6)
4 (FTI-2237)	>30	nd	nd (0.78)
5 (FTI-2220)	5	89.1	>100 (0.26)
6 (FTI-2238)	10	72	nd (0.041)
7 (FTI-2289)	13	86.3	5 (0.0072)
8 (FTI-2277)	3	nd	< 100 (0.38)
9 (FTI-2278)	3	nd	>100 (3.1)
10 (FTI-2279)	4	nd	nd (0.64)
11 (FTI-2291)	10	nd	20 (0.22)
12 (FTI-2148)	>30	0	0.2 (0.001)
13 (FTI-2153)	2	99	0.02 (34)

[a]Inhibition at 20 μM, RBC = red blood cell.

μg/mL. Compounds **5** and **6**, in which the 5-position of the imidazole ring is linked to the hydrophobic 4-aminobenzoate spacer, showed about 2-fold higher activity than corresponding 4-substituted isomers, **3** and **4**. A similar dependence on the substitution positions of the imidazole was seen in the inhibition of mammalian FTase and reflects a well-matched interaction of inhibitors **5** and **6** with the enzyme active site. Interestingly, compound **5** was more than 20 times more active at inhibiting the growth of *P. falciparum* than in blocking protein farnesylation in mammalian cells, reflecting possible differences in FTase inhibition activity and membrane permeation between the two organisms.

The simpler series of non-methionine-containing compounds **8**–**11** also showed potency against *P. falciparum* in the low μg/mL range. These are unlikely to inhibit malarial FTase, and thus probably act by a mechanism other than inhibition of protein prenylation. However, confirmation of this conclusion must await inhibition studies on malarial FTase. Comparison among compounds **8**–**10** and **11**, suggests that larger hydrophobic substituents at the C-terminus of the peptidomimetic increase inhibitory activity (e.g., $ED_{50} = 3.6$ μg/mL for **10**; $ED_{50} = 9.91$ μg/mL for **11**). Compound **12** (FTI-2148), one of the most potent mammalian FTase inhibitors ($IC_{50} = 0.001$ μM), did not inhibit parasite growth, presumably because of poor membrane permeability from the free methionine carboxylate terminus. In contrast, the methyl ester form of **12**, compound **13** (FTI-2153), showed high inhibition activity against growth of *P. falciparum* ($ED_{50} = 2$ μg/mL).

The results shown here suggest that the active site recognition properties of malarial FTase should be different from those of the mammalian enzyme, so that selective inhibition of parasitic growth over mammalian FTase inhibition should be possible.

In summary, we have developed a series of non-thiol FTase inhibitors that are potent inhibitors of the growth of *P. falciparum*. We are continuing to investigate *P. falciparum* FTase as a novel drug target and are evaluating the antiparasitic activity of these and other FTase inhibitors in animal models of malaria.

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