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Structurally Simple Farnesyltransferase Inhibitors Arrest the Growth of Malaria Parasites**

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The development of antimalarial agents is not only constricted by the parameters common to the development of all new drugs (efficacy, toxicity, pharmacology), but importantly, must also recognize the economic reality of the countries most affected. It is estimated that there are 300–500 million acute cases of malaria each year, resulting in 1–3 million deaths, with 90% of the mortality occurring in Africa.[1] The severe poverty in the regions most affected by malaria and the staggering number of people infected necessitate very inexpensive treatment. In an effort to decrease the cost of developing new antimalarial drugs, recent attention has been

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directed at investigating parasitic proteins that are validated therapeutic targets in other diseases, thereby accelerating access to new classes of inhibitors.[2,3]

Recently, prenylated proteins and associated prenyltransferases have been identified in the malarial protozoa *Plasmodium falciparum*.[2] Prenylation is a required modification for the function of a variety of cellular proteins, including small G proteins,[4] heterotrimeric G-protein γ subunits,[5] protein kinases,[6] and nuclear lamins.[7] Studies of the role of prenylation in modulating the function of the GTP-binding proteins Ras and Rho, which have oncogenic forms common to many human cancers (≈30%), have identified the ubiquitous eukaryotic enzyme protein farnesyltransferase (PFTase) as a key target for the interception of aberrant Ras activity.[8] Subsequent efforts to identify small-molecule inhibitors of PFTase have led to several potential anticancer agents currently in clinical trials.[9,10] The application of related PFTase inhibitors to cells infected with *P. falciparum* led to a decrease in farnesylated proteins and to the associated lysis of the parasites.[11] Importantly, the PFTase inhibitors studied were found to be significantly more toxic against the parasite than to mammalian cells. In this study, we developed a new series of PFTase inhibitors as novel antimalarial agents, emphasizing simple molecular architecture and straightforward chemical synthesis as a prerequisite for access to low-cost treatment.

PFTase is one of three closely related heterodimeric zinc metalloenzymes (protein farnesyltransferase and geranylgeranyltransferases I and II) that catalyze the transfer of prenyl groups from farnesyl or geranylgeranyl pyrophosphate to the free thiol group of a cysteine residue within the tetrapeptide recognition sequence CaaX (a = aliphatic amino acid, X is often M, S, A, or Q for PFTase) located at the carboxyl terminus of the substrate protein.[12] A homology model of malarial PFTase (*Pf*PFT) was generated with MODELLER,[13] by using the crystal structure of rat PFT complexed with the nonsubstrate tetrapeptide inhibitor CVFM, and farnesyl diphosphate (FPP) as the template structure (PDB entry 1JCR). The sequences of the two subunits (α and β) of *Pf*PFT were obtained from the PlasmoDB database (gene loci: PFL2050w, α and chr11.glm_528, β)[14,15] and aligned with the template with the program T-COFFEE.[16] Only regions with reasonable reliability in the alignment were included. The model of *Pf*PFT comprises the following sequence segments (the residue numbers of the corresponding segments of the rat PFT subunits are given in parentheses): α: 72–164 (87–179), 300–411 (184–283); β: 421–677 (71–315), 806–896 (330–417). *Pf*PFT and rat PFT share 23% identity and 53% similarity for the α subunit; the respective values for the β subunit are 37 and 56%. The catalytic zinc ion, six structurally conserved water molecules, and FPP were included in the model. The conformation of FPP was considered flexible during the model calculations. For this purpose, the force-field parameters for FPP were added to the MODELLER force field on the basis of the lipid parameters of the charmm27 force field.[17] The model with the lowest value of the objective function of MODELLER from twenty different calculations was used for docking studies.

The homology model indicates a large, open, and predominately hydrophobic cavity for the active site (≈20 × 20×20 Å^3), with the phospholipid binding partner (FPP) extending across the cavity base. The Zn ion coordinates to three residues (Cys661, Asp659, and His838) with a water molecule hydrogen bonded between the terminal phosphate of FPP and Asp659, defining the limit of the Zn binding domain (Figure 1). The remainder of the active-site cavity includes two well-defined hydrophobic pockets (Lys149, Asn317 Ser150, Phe150 and Trp456, Trp452, Tyr837), a larger hydrophilic domain formed by Arg564, and three water molecules participating in a hydrogen-bonded network between Ser449 and Gln152.

Flexible ligand-docking studies (GOLD)[18] indicate that the four pockets of the homology model active site can be efficiently accessed from a simple aliphatic tether, affording PFTase inhibitors with an acyclic scaffold that can be conveniently prepared through a short series of straightforward chemical transformations (Figure 1). One of the simplest scaffolds conceivable, ethylenediamine, provides an inexpensive and fourfold substitutable tether of appropriate size to project four different groups into the active site

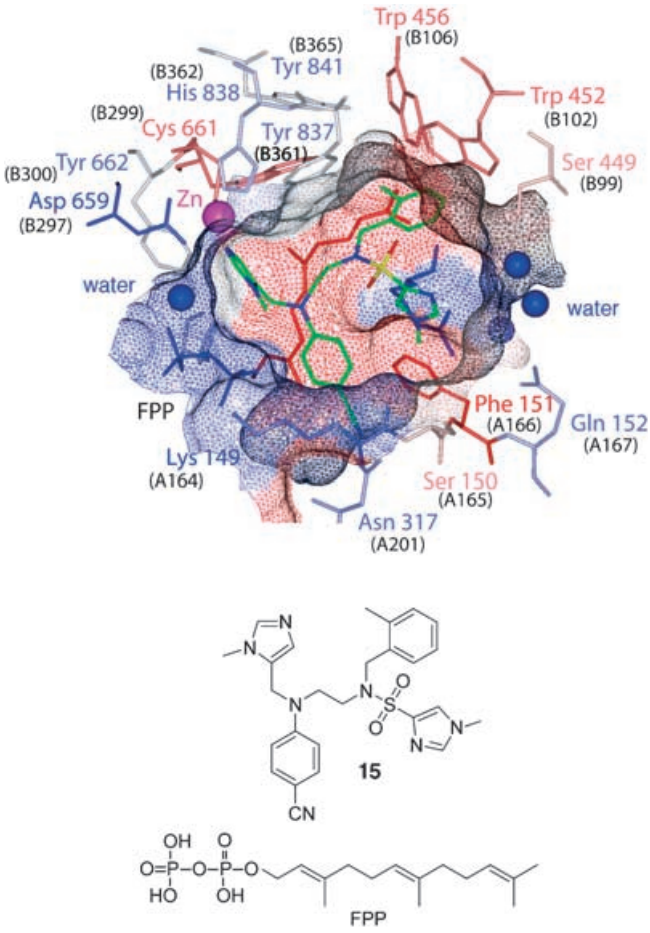

Figure 1. Active-site conformation of **15** (colored by atom type), as determined by flexible ligand docking[18] in the homology model of the active site of *Pf*PFT (red = hydrophobic, blue = hydrophilic residues). Values in parentheses refer to the corresponding positions in rat PFT (PDB entry 1JCR).

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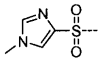
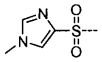
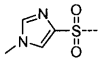
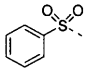
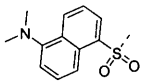
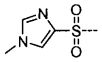
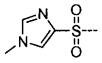
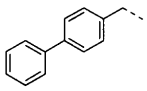
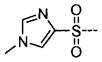
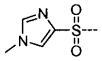
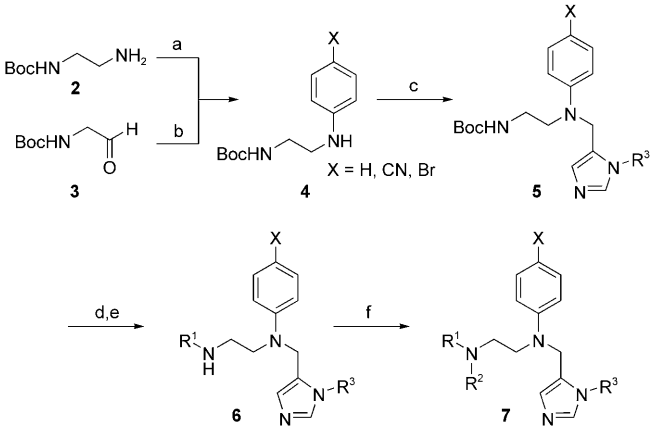
pockets. The incorporation of imidazole provides a zinc binding group that has been consistently demonstrated to confer activity in other series of inhibitors, whereas small hydrophobic (phenyl, benzyl) and simple sulfonamides (*N*-methylimidazole, phenyl) are predicted as suitable substitutions to access the remaining hydrophilic and hydrophobic pockets.

An initial series of PFTase inhibitors was prepared as outlined in Scheme 1. Substituted anilines **4** were formed either by the reductive amination of aniline (4-H, 4-Br), or by nucleophilic substitution of *para*-fluorobenzonitrile with monoprotected ethylenediamine. Chemoselective alkylation of aniline **4** through double deprotonation with LDA and subsequent alkylation at the anilide anion proceeds smoothly in THF with no evidence of alkylation of the carbamate. Some cyclic urea is observed, presumably generated through the elimination of *tert*-butoxide from the carbamate to provide the corresponding isocyanate and subsequent intramolecular cyclization to the anilide. Alkylation of carbamate **5**, deprotection, and coupling to sulfonyl chlorides furnishes the desired series of inhibitors (**1**, **8**–**15**, Table 1).

Table 1: Activity of acyclic ethylene diamine compounds against *Pf*PFT and parasite-infected cells.

Number	X	Compound R^1	R^2	R^3	IC_{50} [nM][a] *Pf*PFT	ED_{50} [nM][b] 3D7	K1
8	H			H	215	ND[c]	ND[c]
9	Br			CH_3	2.0	675	3200
1	CN			CH_3	0.5	349	375
10	CN			CH_3	8.9	2500	2250
11	CN			CH_3	55	300	388
12	CN			CH_3	4.5	220	850
13	CN			CH_3	>1000	2800	>5000
14	CN			CH_3	240	700	2500
15	CN			CH_3	0.6	93	150

[a] Inhibitor concentration required to decrease *Pf*PFT activity twofold. [b] Inhibitor concentration required to decrease hypoxanthine incorporation into parasites twofold. [c] Not determined.



Scheme 1. Conditions: a) 4-fluorobenzonitrile, TEA, DMSO, 120 °C; b) 4-bromoaniline, $NaCNBH_3$, acetic acid, molecular sieves (3 Å), MeOH; c) LDA, NaH, 5-chloromethyl-1-methyl-1*H*-imidazole·HCl (R^3), THF, −78 °C→RT; d) NaH, alkyl bromide (R^1), DMF, 0 °C; e) TFA; f) arylsulfonyl chloride (R^2), TEA, DMF. Boc = *tert*-butoxycarbonyl, DMF = *N*,*N*-dimethylformamide, LDA = lithium diisopropylamide, TEA = triethylamine, TFA = trifluoroacetic acid.

A focused set of diversity elements, predicted by docking studies (GOLD)[18] to efficiently occupy the four binding domains of the active site, were applied in the preparation of nine compounds, and their inhibition of *Pf*PFT was determined by using a scintillation proximity assay with partially purified *Pf*PFT (Table 1).[19] Eight of the nine compounds inhibit *Pf*PFT at concentrations of 50 nM or lower, with four compounds having IC_{50} values below 5 nM (**1**, **9**, **12**, and **15**). The growth of parasites in whole cells (3D7, K1) treated with inhibitors was monitored through the incorporation of tritium-labeled hypoxanthine. Six compounds displayed ED_{50} values below 1 μM, with compound **15** showing significant whole-cell activity (ED_{50}: 3D7, 93 nM; K1, 150 nM). The significantly improved activity of compounds **1** and **9**, relative to that of **8**, reflects docking solutions that suggest the *para* substituent of aniline (X) contacts a hydrophilic domain formed by Ser150, Asn317, and Lys149, whereas methylation of the zinc binding group complements a small adjacent hydrophobic pocket. Of the four substitutions investigated for the R^1 hydrophobic pocket, benzyl and cyclohexylmethyl groups afforded best activity, whereas *tert*-butylcarbamate and 4-phenylbenzyl groups are significantly less active. Three substitutions were assessed for the hydrophilic cavity formed predominately by Arg564. The small basic 1-methyl-1*H*-imidazole-4-sulfonamide conferred the best potency against both the enzyme and in whole-cell assays, whereas dansyl sulfonamide afforded significantly lower enzyme inhibition, but retained good potency (ED_{50} 300 nM) against whole cells.

Importantly, these early examples of a new series of simple acyclic *Pf*PFT inhibitors demonstrate their reasonable metabolic stability, and oral bioavailability. Treatment of **15** with rat liver microsomes resulted in a steady transformation of the inhibitor over one hour ($t_{1/2} = 20$ min), with principal metabolites observed by MS corresponding to oxidation of the inhibitor (+O), and loss of the aniline imidazole (R^3, data not shown). The promising metabolic stability of this series of inhibitors is further reflected by the reasonable plasma

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concentration of inhibitor observed after oral dosing. Oral gavage of rats with **15** induced peak plasma concentrations of inhibitor 10-fold above the ED_{50} value within 30–40 min. A plasma concentration at least fivefold above ED_{50} was maintained for approximately two hours, with inhibitor clearing observed ($t_{1/2} \approx 33$ min, Figure 2).

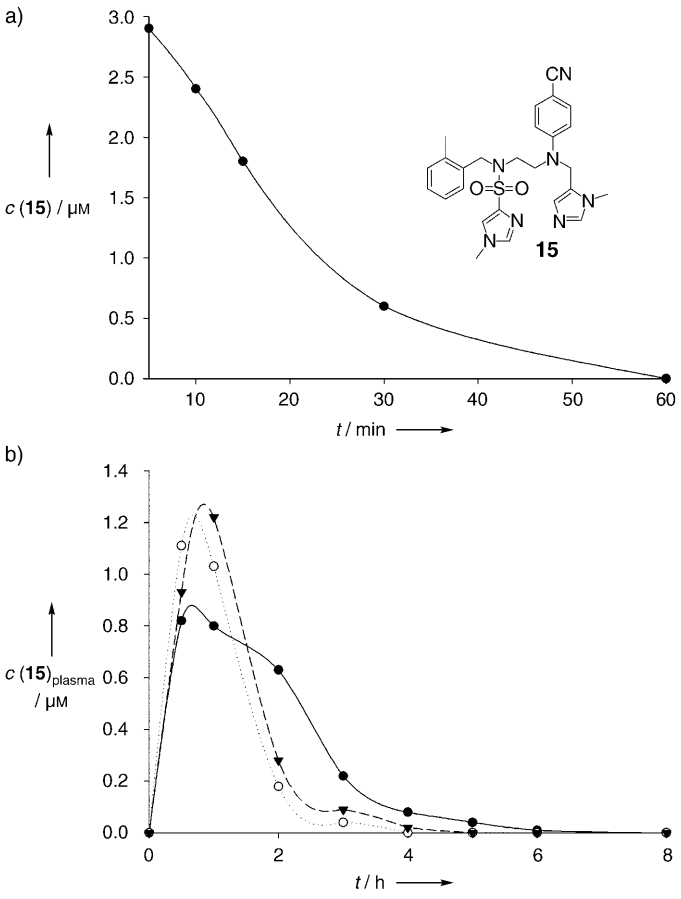

Figure 2. a) Metabolism of **15** by rat liver microsomes ($t_{1/2} = 20$ min); b) inhibitor concentration in plasma of **15** in three rats after oral garage (○ = rat 1, ▼ = rat 2, ● = rat 3; dose: 12.5 mg kg^{-1}, $t_{1/2} \approx 33$ min, $c_{max} = 1.05 \pm 0.21$ μM, $t_{max} = 40 \pm 17$ min).

In summary, a new series of simple acyclic *Pf*PFT inhibitors was developed, and their potency evaluated in enzyme and whole-cell assays. Compounds based on this readily accessible scaffold are highly active inhibitors of *Pf*PFT, with promising oral bioavailability. Expansion of this inhibitor series is expected to provide potent, orally available compounds as promising new low-cost drugs for the treatment of malaria.

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