Novel *N*-(4-Piperidinyl)benzamide Antimalarials with Mammalian Protein Farnesyltransferase Inhibitory Activity

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Protein farnesyltransferase of *Plasmodium falciparum* is a potential target in the treatment of malaria for which increased drug resistance is observed. The design, synthesis and evaluation of a series of *N*-(4-piperidinyl)benzamides is reported. The most potent compounds showed *in vitro* activity against the parasite at submicromolar concentrations.

Key words farnesyltransferase; malaria; *Plasmodium falciparum*; *N*-(4-piperidinyl)benzamide derivative; inhibitor

Antimalarial drugs saved millions of lives during the 20th century. However, owing to the onset of drug resistance, there is now an urgent need to identify new drug targets in *Plasmodium falciparum* and to develop effective new antimalarial agents.[1,2] Prenylated proteins have been shown to function in important cellular processes including signal transductions. Among them, protein farnesyl transferase (PFT) has been a major target in the conception of new anticancer drugs.[3]

In an effort to identify a new and more effective drug target, Chakrabarti[4,5] found that the peptidomimetic L-745,631 (Chart 1) was the best inhibitor of *P. falciparum* PFT and also a good inhibitor of parasite growth.

This finding suggests the real potential of designing or identifying inhibitors of *P. falciparum* prenyl transferase as an approach to malaria therapy. In addition, several works have reported mammalian PFT inhibitors displaying potent antimalarial activities *in vitro* and more recently *in vivo*.[6] Small inhibitors of mammalian FTase developed as anticancer drugs like BMS-214662[7—11] are also effective inhibitors of *P. falciparum* growth.[12,13]

Results and Discussion

Our work has focused on peptidomimetic inhibitors based on the CA_1A_2X tetrapeptide, known to be responsible for interaction with the mammalian FTase, where the A_1A_2 peptide is replaced by the structurally restricted *N*-(4-piperidinyl)benzamide scaffold. Initial studies[14] led us to compound **1** which possessed an IC_{50} (isolated enzyme FTase) as low as 22.8 nM, but did not inhibit the proliferation of tumor cells in culture (Chart 2). More recently,[15] we synthesized a series of derivatives of compound **1**, of general structure **2** which was the outcome of three structural modifications: (i) replacement of methioninate by phenylalaninate or isoleucinate (R_1) with the aim of increasing selectivity *versus* geranylgeranyl transferase, based on our previous results[14] (ii) replacement of the cysteinyl moiety by a known metal chelator, *i.e.* (1-benzylimidazol-5-yl)methyl substituted in *para* position (R_2) of benzyl in order to increase cellular uptake (iii) reduction of the benzoyl group into benzyl (R_3) in order to introduce flexibility into this region of the molecule. Target compounds **15a**—**c**, **16c**, **17c**, **18** were obtained by reductive amination between adequate imidazolylcarbaldehydes **8a**—**c** and 4-aminopiperidines **12**—**14**. The synthesis of 5-formylimidazoles **8a**—**c** was completed (Chart 3) in 5 steps according to a strategy[16,17] described for the preparation of regiochemically substituted imidazoleacetic esters: tritylation of N_1-imidazole (**4**), protection of primary alcohol as ester (**5**), benzylation of N_3 and detritylation of N_1 (**6a**—**c**). The aldehyde function was finally created by chemical oxidation of the hydroxymethyl group (**7a**—**c**) resulting from hydrolysis of the ester (**6a**—**c**). Piperidines **9**—**11** were also prepared (Chart 4) from reductive amination of *N*-Boc-piperidone with adequate α-amino esters. Benzoylation and deprotection of piperidine (compounds **12**—**14**, **18**), possibly followed by reduction of benzoyl into benzyl for **12**, was the last step before aminative reduction which yielded final compounds **15**—**17** and **19**.

Antimalarial activities (Table 1) of piperidines **1**, **15a**—**c**, **16c**, **17c**, and **19** range from 4.4 to 0.85 μM with submicromolar activity for the most potent compound, **15b**. Introduction of a trifluoromethyl substituent into the para of the benzyl group increases potency about 3-fold when compared to the non substituted compound **15a**. The nature of the benzyl

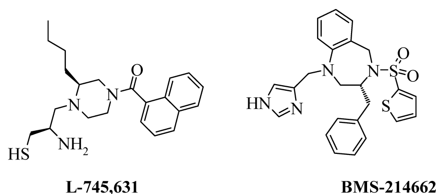

Chart 1

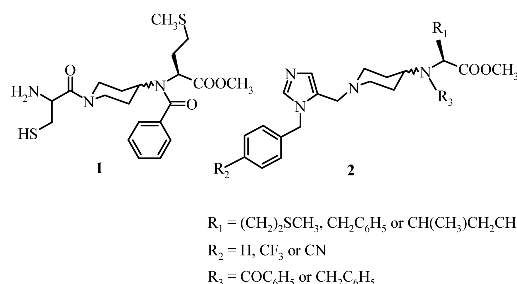

Chart 2

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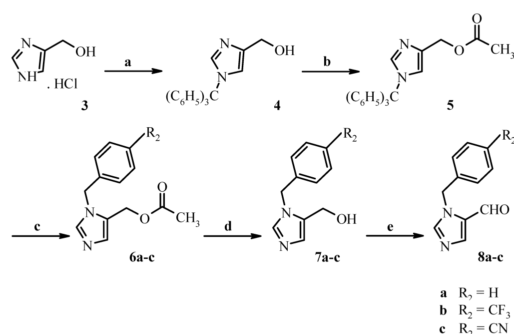
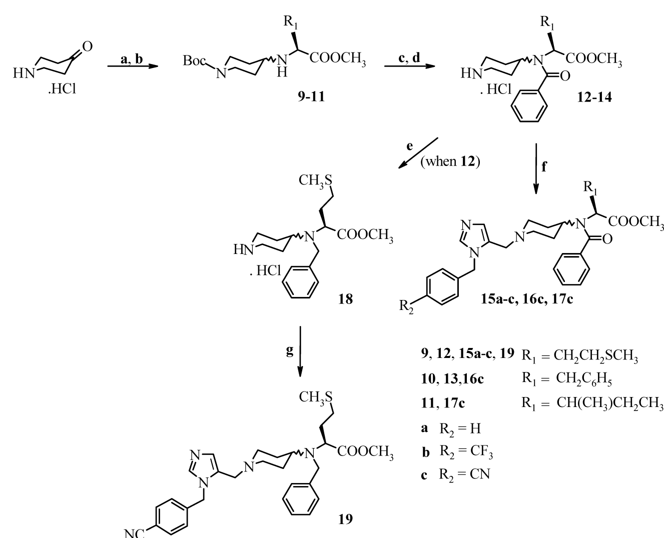


Reagents and conditions: (a) $(C_6H_5)_3CCl$, NEt_3, DMF, rt, 72 h, 71%; (b) $(CH_3CO)_2O$, pyridine, rt, 18 h, 84%; (c) i) $(p)R_2$-$C_6H_4CH_2Br$, EtOAc, 55 °C, 24 h; ii) MeOH, reflux, 18 h, 69—77% for **6a**, **b** or TFA, rt, 1 h, 57% for **6c**; (d) 2 N NaOH, MeOH, rt, 0.5—2 h, 36—99%; (e) MnO_2, dioxane, 30—40 °C, 3 h, 70—97%.

Chart 3



Reagents and conditions: (a) Boc_2O, DIEA, dioxane/H_2O (4 : 1), rt, 24 h, 75%; (b) H-(Met, Phe or Ile)-OCH_3, NEt_3, $NaBH_3CN$, MeOH, 50 °C, 48 h, 40—68%; (c) Benzoyl chloride, NEt_3, CH_2Cl_2, 0 °C, 24 h, 30—55%; (d) HCl/MeOH, rt, 18 h, 95%; (e) i) BH_3.THF, THF, 0 °C, 18 h; ii) 6 N HCl, H_2O, reflux, 10 min, 30%; (f) i) **8a**—**c**, NEt_3, MeOH; ii) $NaBH_3CN$, MeOH, 50 °C, 18 h, 42—52%; (g) i) **8c**, NEt_3, MeOH; ii) $NaBH_3CN$, MeOH, 50 °C, 18 h, 47%.

Chart 4

substituent is important, as replacement of CF_3 by CN lowers potency about 3-fold (compound **15c**). This result suggests that lipophilicity at this region of the molecule could have an important role in antimalarial activity. Replacement of the benzyl-4*H*-imidazol-4-ylmethyl moiety of compound **15a** by a cysteinyl residue (compound **1**) at the piperidinyl nitrogen increased antimalarial activity, which could also be attributed to stronger lipophilicity of the imidazolyl fragment compared to that of the cysteinyl one. Replacement of methioninate in compound **15c** by other amino acid residues such as phenylalaninate (**16c**) or isoleucinate (**17c**) led to an increase in antimalarial activity, as high as 2-fold in the case of **17c**. Aromaticity and greater lipophilicity in this region of the molecule seem to be favourable to antimalarial potency. Reduction of benzamide into benzylamine decreases antimalarial activity (**15c** *vs.* **19**). As previously seen for other series of FTase mammalian inhibitors with potent antimalarial activities,[4,18,19] no correlation can be observed between the two biological activities. The most potent inhibitor of FTase in the series (**19**) displays the lowest antimalarial activity. On the other hand, the most potent antimalarial derivative, **15b**, also displays good inhibition activity on FTase whereas compound **15a**, possessing the lowest FTase inhibition, also displays low antimalarial activity. Taken together, these results suggest that in the set of compounds reported here, inhibitory activity of mammalian FTase is not sufficient to explain the antimalarial activities observed. Several works[16,18,19] suggest that the active-site recognition properties of malarial FTase should be different from those of the mammalian enzyme. This difference could partly account for the lack of correlation noted between the two activities. However, a more recent work[25] suggests that the active-site residues of the farnesyltransferase of *P. falciparum* and rat are nearly identical despite low overall sequence identity due to several additional external loops of the *P. falciparum* enzyme. Except for structural microscopic differences between the two enzymes, there are other factors which could account for the differences noted. Pharmacological properties such as lipophilicity or weak basic character could be involved in cellular uptake inside the parasite. Additional mechanisms cannot be excluded such as complexation of imidazole moiety with ferriproto-

Table 1. Biological Evaluation of Compounds **1**, **15a**—**c**, **16c**, **17c**, and **19**

Compd.	R_1	R_2	R_3	IC_{50} [a,b] (nM)		IC_{50} [22] (μM) *P. falciparum*
				FTase[c]	GGTase-1[c]	
CQ[d]	—	—	—	Nd[e]	Nd	0.13±0.03
1	—	—	—	22.8±2.1	>100	1.6±0.3
15a	$(CH_2)_2SCH_3$	H	COC_6H_5	397±84	Nd	2.9±0.9
15b	$(CH_2)_2SCH_3$	CF_3	COC_6H_5	43.9±4.2	Nd	0.85±0.14
15c	$(CH_2)_2SCH_3$	CN	COC_6H_5	4.60±2.36	>2000	3.0±0.9
16c	$CH_2C_6H_5$	CN	COC_6H_5	22.0±6.6	>2000	7.0±0.1
17c	$CH(CH_3)CH_2CH_3$	CN	COC_6H_5	32.7±3.8	>2000	1.4±0.4
19	$(CH_2)_2SCH_3$	CN	$CH_2C_6H_5$	2.35±0.48	>2000	4.4±0.1

a) Values are means of three determinations. *b*) See refs. 15, 20, 21. *c*) Mammalian enzyme. *d*) Chloroquine. *e*) Not determined.

Table 2. Antiproliferative Effects of 4-Aminopiperidine FTIs on L-1210 and DLD-1 Cell Lines

Compd.	IC_{50}[a] (μM)	
	L-1210	DLD-1
1	>10 (>6.2)[b]	18.1±2.3 (11)
15a	>10 (3.5)	27.1±6.6 (9.3)
15b	>10 (>12)	63.1±18.6 (74)
15c	0.020±0.006 (0.007)	9.20±2.47 (3.0)
16c	4.95±0.29 (0.7)	7.74±3.62 (1.6)
17c	6.62±0.32 (4.7)	26.5±6.3 (19.0)
19	3.95±0.22 (0.9)	12.9±3.8 (2.9)

a) See ref. 27. *b*) The ratio $IC_{50\ compound}/IC_{50\ P. falciparum}$ is given in parentheses.

porphyrin IX (FP) reported for potent *in vitro* imidazole antimalarials,[26] which could also help to explain the differences noted.

Cytotoxicities on mammalian cells (Table 2) were evaluated on two selected cell lines (murine leukemia cell line L-1210 and human colon cell line DLD-1 which expresses K-Ras isoform in wild type) in order to determine the ratio between antiproliferative effects of compounds and *in vitro* activity against *P. falciparum*. The compounds have IC_{50} values against L-1210 and DLD-1 in the micromolecular range, except for **15c** (on L-1210). As seen with the direct comparison of the IC_{50} values, there is no significant relationship. For example, **15b** has better activity for *P. falciparum* than for L-1210 and DLD-1 cell lines, which was not observed for **15c**. This shows that it is possible to develop antimalarial drugs without cytotoxic side effects.

In conclusion, new *N*-(4-piperidinyl)benzamide derivatives have been designed as FTase mammalian inhibitors displaying significant antimalarial micromolar and submicromolar activities. The results obtained demonstrate that requirements for FTase inhibition and antimalarial activity on the *N*-(4-piperidinyl)benzamide core are not exactly similar, suggesting that selective inhibition of parasitic growth over mammalian FTase inhibition should be possible. This study enables us to highlight a new antimalarial lead compound, **15b**. Further modulations to this compound are ongoing in order to optimize its antiparasitic activity. The first goal consists in combining the presence of diverse hydrophobic and bulky R_1 substituents with the replacement of methionine by isoleucine or other lipophilic groups. This work, together with other previous studies, shows that despite the absence of recombinant *P. falciparum* enzyme, the screening of FTase mammalian inhibitors on parasite growth can provide a number of new antimalarial leads.

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