Inhibition of *Plasmodium falciparum* Fatty Acid Biosynthesis: Evaluation of FabG, FabZ, and FabI as Drug Targets for Flavonoids

Deniz Tasdemir,*[,†,||] Gabriela Lack,[§] Reto Brun,[‡] Peter Rüedi,[†] Leonardo Scapozza,[§] and Remo Perozzo*[,§]

University of Zurich, Institute of Organic Chemistry, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland, Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland, and Pharmaceutical Biochemistry Group, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland

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After the discovery of a potent natural flavonoid glucoside as a potent inhibitor of FabI, a large flavonoid library was screened against three important enzymes (i.e., FabG, FabZ, and FabI) involved in the fatty acid biosynthesis of *P. falciparum*. Although flavones with a simple hydroxylation pattern (compounds **4**−**9**) showed moderate inhibitory activity toward the enzymes tested (IC_{50} 10−100 μM), the more complex flavonoids (**12**−**16**) exhibited strong activity toward all three enzymes (IC_{50} 0.5−8 μM). Isoflavonoids **26**−**28** showed moderate (IC_{50} 7−30 μM) but selective activity against FabZ. The most active compounds were C-3 gallic acid esters of catechins (**32**, **33**, **37**, **38**), which are strong inhibitors of all three enzymes (IC_{50} 0.2−1.1 μM). Kinetic analysis using luteolin (**12**) and (−)-catechin gallate (**37**) as model compounds revealed that FabG was inhibited in a noncompetitive manner. FabZ was inhibited competitively, whereas both compounds behaved as tight-binding noncompetitive inhibitors of FabI. In addition, these polyphenols showed in vitro activity against chloroquine-sensitive (NF54) and -resistant (K1) *P. falciparum* strains in the low to submicromolar range.

Introduction

Malaria is one of the most important parasitic diseases in the world, and its impact has started to increase during the past decade.[1] An effective vaccine is still not available, and the antimalarial treatment depends on drugs, which loose efficacy because of emerging drug resistance. Consequently, there is a need to find new lead compounds against established or new drug targets. A promising recently discovered target is the type-II fatty acid biosynthesis (FAS-II)[a] pathway of *P. falciparum*, and it has been shown that FAS-II can be exploited for antimalarial drug discovery.[2−6] A series of unique enzymes are involved in the FAS-II pathway, among which are β-ketoacyl-ACP-reductase (FabG), β-hydroxacyl-ACP-dehydratase (FabZ), and enoyl-ACP-reductase (FabI).

Flavonoids comprise a large group of polyphenolic secondary metabolites that are widespread throughout the plant kingdom, ranging from mosses to angiosperms.[7] They are all based on the flavan skeleton, consisting of two aromatic rings (A and B) interconnected by a three carbon atom heterocyclic ring C (Figure 1). Depending on the modifications of ring C, they can be classified into six main groups, that is, flavanones, flavones, isoflavones, flavonols, flavanols, and anthocyanins. In plants, flavonoids occur in different forms, such as free aglycones, glycosides, and biflavonoids. The polyphenolic structure allows a large number of further substitutions, including phenolic hydroxy groups, methoxy groups, O-sugars, C-sugars, and sulfates, thus producing an extremely diverse range of derivatives. To date, more than 6000 different flavonoids have been described, and the number continues to increase.[8]

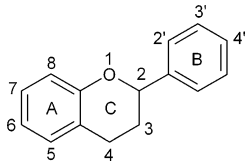

Figure 1. Basic flavonoid structure. Depending on the modifications on ring C, flavonoids are classified into main groups of flavanones, flavones, isoflavones, flavonols, flavanols, catechins, and anthocyanins.

Flavonoids are ubiquitously found in foods (fruits and vegetables) and beverages (red wine, tea, and juices) and, therefore, are common components of the human diet. They exert a number of biological activities, such as antiviral, antibacterial, antiprotozoal, oestrogenic, antiinflammatory, mutagenic, antimutagenic, and antineoplastic activities, and are also capable of inhibiting many types of enzymes.[8−10] Various epidemiological studies indicate that a flavonoid-rich diet is associated with a lower incidence of cancer and cardiovascular diseases,[11,12] probably because of their antioxidant[13,14] and vasoactive properties.[15,16] Several flavonoids and green-tea catechins, particularly (−)-epigallocatechin gallate (**33**), have been demonstrated to inhibit the fungal as well as the human type-I fatty acid biosynthesis system.[17−19] Recently, it was shown that **33** and related flavonoids are potent inhibitors of *Escherichia coli* FabG and FabI, two important reductases involved in chain elongation of type-II fatty acid biosynthesis in bacteria.[20]

In a recent article, we reported a common flavonoid glycoside, luteolin-7-*O*-glucoside, to be the first antimalarial natural product targeting the FabI enzyme of *P. falciparum*.[21] This study prompted us to investigate the inhibitory activity of a large flavonoid library against FabI as well as two other crucial enzymes (FabG and FabZ) of the type-II FAS system of *P. falciparum* and determine the in vitro antiplasmodial potential of these compounds. Two compounds, luteolin (**12**) and (−)-catechin gallate (**37**) were subjected to further kinetic investiga-

* To whom correspondence should be addressed. Phone: +44-(0)20-7753-5845. Fax: +44-(0)20-7753-5909. E-mail: deniz.tasdemir@pharmacy.ac.uk, dtasdemir@hotmail.com (D.T.). Phone: +41-22-3793371. Fax: +41-22-3793360. E-mail: remo.perozzo@pharm.unige.ch (R.P.).

† Institute of Organic Chemistry.

‡ Swiss Tropical Institute.

§ University of Geneva.

|| Current address: Department of Pharmaceutical and Biological Chemistry, Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX.

[a] Abbreviations: FAS-I, type I fatty acid synthase; FAS-II, type II fatty acid synthase; ACP, acyl carrier protein; FabG, β-ketoacyl-ACP reductase; FabZ, β-hydroxyacyl-ACP dehydratase; FabI, enoylacyl-ACP reductase; DMSO, dimethyl sulfoxide;

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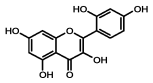
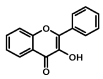
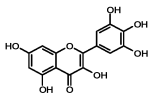
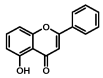
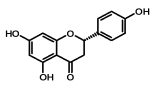
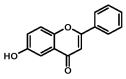
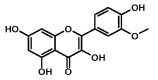
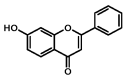
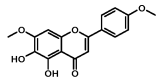
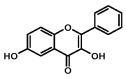
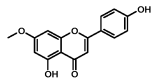
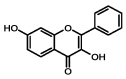
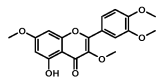
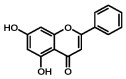
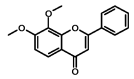
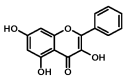
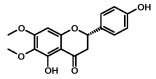
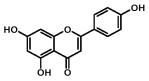
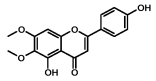
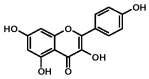
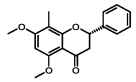
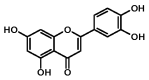
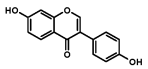
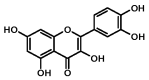
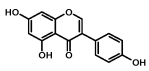
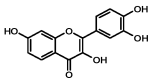
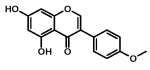
Table 1. Inhibition of *P. falciparum* Enzymes FabG, FabZ, FabI, and Chloroquine-Sensitive (NF54) and -Resistant (K1) *P. falciparum* Strains by Flavonoids

			IC_{50} (μM)								IC_{50} (μM)				
compd	name		FabG	FabZ	FabI	P.f. NF54[a]	P.f. K1	compd	name		FabG	FabZ	FabI	P.f. NF54	P.f. K1
1	flavone		n.a.[b]	n.a.	n.a.	60.8	n.d.[c]	**15**	morin		2.3	8	5	110.4	16.1
2	3-hydroxy-flavone		n.a.	n.a.	n.a.	24.0	n.d.	**16**	myricetin		14	2	0.4	57.3	12.9
3	5-hydroxy-flavone		n.a.	n.a.	n.a.	65.9	n.d.	**17**	naringenin[d]		n.a.	n.a.	n.a.	141.1	n.d.
4	6-hydroxy-flavone		n.a.	30	n.a.	5.3	n.d.	**18**	isorhamnetin		8.3	n.a.	5	14.7	n.d.
5	7-hydroxy-flavone		n.a.	20	n.a.	65.5	n.d.	**19**	ladanein		n.a.	n.a.	n.a.	9.0	n.d.
6	3,6-dihydroxy-flavone		n.a.	n.a.	n.a.	36.4	n.d.	**20**	genkwanin		n.a.	n.a.	n.a.	111.0	n.d.
7	3,7-dihydroxy-flavone		n.a.	n.a.	10	49.2	n.d.	**21**	quercetin-3,7,3',4'-tetramethylether		n.a.	n.a.	n.a.	73.3	n.d.
8	chrysin		10	n.a.	n.a.	29.8	n.d.	**22**	7,8-dimethoxy-flavon		n.a.	n.a.	n.a.	40.9	n.d.
9	galangin		100	n.a.	n.a.	39.4	n.d.	**23**	5,4'-dihydroxy-6,7-dimethoxy-flavanone[d]		n.a.	n.a.	n.a.	8.8	n.d.
10	apigenin		n.a.	n.a.	50	17.4	n.d.	**24**	cirsimaritin		70	n.a.	n.a.	16.9	n.d.
11	kaempferol		4	n.a.	20	30.4	n.d.	**25**	5,7-dimethoxy-8-methyl-flavanone[d]		n.a.	40	n.a.	19.8	n.d.
12	luteolin		4	5	2	10.7	9.6	**26**	daidzein		n.a.	30	n.a.	103.7	n.d.
13	quercetin		5.4	1.5	1.5	10.0	8.9	**27**	genistein		n.a.	7	n.a.	38.2	n.d.
14	fisetin		4.1	2	1	8.2	6.5	**28**	biochanin A		n.a.	8	n.a.	34.3	n.d.

[a] Artemisinin was used as the reference standard (IC_{50}: 0.002 μg/mL). [b] n.a. means that the compound was not active when measured at a concentration of 100 μM. [c] n.d. means not determined. [d] The compound exhibits an S configuration at C-2 (2S).

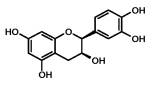
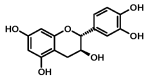
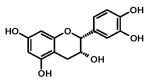
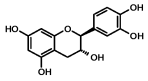
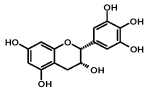
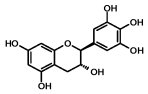
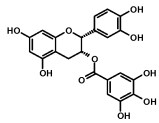
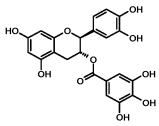
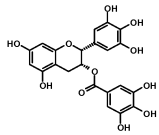
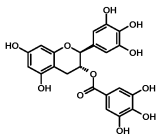
tion to shed light on their inhibitory mechanisms. The current study suggests that flavonoids and analogues are promising antimalarial agents, thus adding up new targets to the broad spectrum biological activities of these compounds.

Results

Inhibition of *P. falciparum* FabG, FabZ, and FabI by Polyphenols. Recently, we have shown that a common flavonoid glycoside, luteolin-7-*O*-glucoside, inhibits FabI, a crucial enzyme involved in fatty acid biosynthesis of *P. falciparum*.[21] After discovering that luteolin (**12**), the aglycone of this compound, exhibits a 20-fold improved inhibition potential, we started to investigate the inhibition activity of a large flavonoid library against three important enzymes of the type-II FAS system of *P. falciparum* (FabG, FabZ, and FabI). The inhibition effects of a variety of polyphenolic compounds against the three enzymes are summarized in Tables 1 and 2.

Several compounds were found to have very good activity against all three enzymes. The flavones and flavonols (= flavon-3-ols) exhibiting a simple substitution pattern, that is, no hydroxy

Table 2. In Vitro Activity of Catechins against FabG, FabZ, FabI and Chloroquine-Sensitive (NF54) and -Resistant (K1) *P. falciparum* Strains

			IC_{50} (μM)								IC_{50} (μM)				
compd	name		FabG	FabZ	FabI	P.f. NF54[a]	P.f. K1	compd	name		FabG	FabZ	FabI	P.f. NF54	P.f. K1
29	(+)-epicatechin		n.a.[b]	n.a.	n.a.	>150	n.d.[c]	**34**	(+)-catechin		n.a.	n.a.	n.a.	144.0	n.d.
30	(-)-epicatechin		n.a.	n.a.	n.a.	>150	n.d.	**35**	(-)-catechin		n.a.	n.a.	n.a.	>150	n.d.
31	(-)-epigallocatechin		n.a.	n.a.	n.a.	>150	n.d.	**36**	(-)-gallocatechin		n.a.	n.a.	n.a.	>150	n.d.
32	(-)-epicatechin gallate		1.0	0.4	0.2	40.0	11.3	**37**	(-)-catechin gallate		1.0	0.4	0.3	3.2	0.4
33	(-)-epigallocatechin gallate		0.3	0.4	0.2	25.5	9.9	**38**	(-)-gallocatechin gallate		1.1	0.6	0.5	40.1	6.2

[a] Artemisinin was used as the reference standard (IC_{50}: 0.002 μg/mL). [b] n.a. means that the compound was not active when measured at a concentration of 100 μM. [c] n.d means not determined.

groups at ring B (for ring definition see Figure 1) and one or two hydroxy groups on rings A/C (Table 1, compounds **1**−**9**) show moderate inhibition effects only toward FabG (**7**, **9**; 10−100 μM), FabZ (**4**, **5**; 20−30 μM), and FabI (**8**; 10 μM). Interestingly, the substitution pattern confers some selectivity for the three enzymes, chrysin (**8**) and galangin (**9**) only having activity against FabG, 6-hydroxyflavone (**4**), and 7-hydroxyflavone (**5**) inhibiting only FabZ, and 3,7-dihydroxyflavone (**7**) exclusively inhibiting FabI. The situation changes with the introduction of hydroxy groups on ring B (Table 1, compounds **10**−**16**). Except for apigenin (**10**; only active against FabI), the above-mentioned selectivity vanishes as the more complex (polyhydroxylated) flavonoids become about 10 times more active and show inhibition of two or all three enzymes at the same time. At this stage, control experiments were carried out to determine potential physicochemical incompatibilities of substrates, cofactors, and compounds and the stability of flavonoids under assay conditions. They were negative, and thus, we could rule out any influence of the experimental conditions on the observed results. Activity against all three enzymes is observed for **12**, quercetin (**13**), fisetin (**14**), morin (**15**), and myricetin (**16**), all of which exhibit IC_{50} values in the range of 0.4−14 μM. The methylation of any of the hydroxy groups in the compounds (Table 1, compounds **18**−**25**) abolishes almost all activity against all three enzymes, with the exception of isorhamnetin (**18**), which remains active against FabI and FabG (IC_{50} of 5 and 8.3 μM, respectively). Among the flavanones tested here (**17**, **23**, and **25**), only 5,7-dimethoxy-8-methylflavanon (**25**) shows any inhibitory activity against FabZ (40 μM). The isoflavonoids tested in this study (Table 1, compounds **26**−**28**) show moderate activity only against FabZ with IC_{50} values in the range of 7−30 μM.

Another interesting group is represented by the catechin-type flavanols that lack both the double bond and the ketone function at ring C (Figure 1). The catechins esterified with gallic acid at position C-3, that is, (−)-epicatechin gallate (**32**), **33**, **37**, and (−)-gallocatechin gallate (**38**), are potent inhibitors of all three enzymes with IC_{50} values in the range of 0.2−1.1 μM (Table 2). Catechins and epicatechins, carrying a free hydroxy group at C-3, neither inhibit the enzymes nor have antiplasmodial activity (Table 2). Gallic acid and other similar phenolic compounds are also devoid of any enzyme inhibitory activity (data not shown).

To elucidate the mechanism and inhibition against the three enzymes, luteolin (**12**) and (−)-catechin gallate (**37**) were chosen as representative compounds. Flavonoid **12** shows IC_{50} values of 4 and 5 μM against FabG and FabZ, respectively. The inhibition of FabI depends on the enzyme concentration used in the assay. For 22 nM FabI an IC_{50} value of 2 μM is observed. Compound **37** inhibits FabG with an IC_{50} value of 1 μM and FabZ with 0.4 μM. Inhibition of FabI by **37** is dependent on the enzyme concentration, as was the case for **12**. The IC_{50} value for **37** is 0.3 μM when 22 nM FabI is used.

Inhibition of FabG by 12 and 37. Compounds **12** and **37** are identified as noncompetitive inhibitors of FabG with respect to acetoacetyl-CoA as well as NADPH (Figure 2). For **37**, the analysis of the data by Dixon plots and secondary plots provides K_i values of 6.4 ± 0.8 μM when varying the acetoacetyl-CoA concentration and 2.8 ± 0.1 μM when varying the NADPH concentration. For **12**, the K_i values were found to be 1.8 ± 0.8 and 0.8 ± 0.1 μM, respectively (Table 3).

Inhibition of FabZ by 12 and 37. An analysis of the inhibition mechanism of FabZ by double reciprocal plots classifies **12** (Figure 3A) and **37** (Figure 3B) as competitive inhibitors of the substrate crotonoyl-CoA. FabZ inhibition by **12** and **37** was analyzed by a Dixon plot. The K_i values were determined from the negative *x*-axis value at the point of intersection of the lines from the slope versus inhibitor replot.

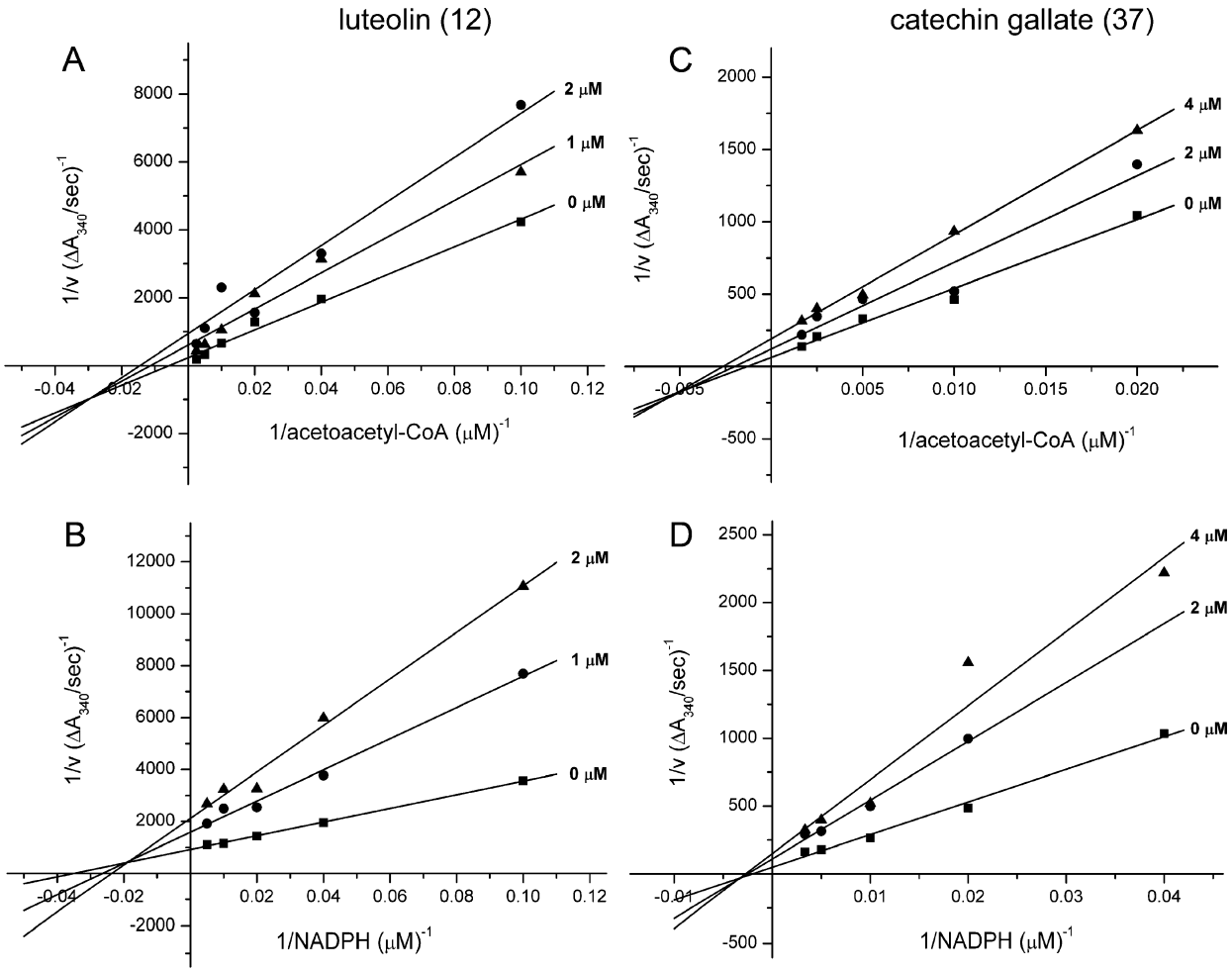


Figure 2. Kinetic analysis of the inhibition mechanism of **12** and **37** on FabG. The four panels show representative plots of $1/v$ vs 1/[acetoacetyl-CoA] (panels A and C) and $1/v$ vs 1/[NADPH] (panels B and D) at different inhibitor concentrations. Compound **12** behaves like a noncompetitive inhibitor (mixed type) with respect to the substrate (panel A) and cofactor (panel B), exhibiting an α value of 0.4 and 1.4, respectively. Compound **37** is a noncompetitive (mixed type) inhibitor of FabG with respect to the substrate (panel C, $\alpha = 0.5$) as well as the cofactor (panel D, $\alpha = 0.8$). The factor α describes the effect of the inhibitor on the affinity of the substrate toward the enzyme and the effect of the substrate on the inhibitor affinity for the enzyme.[22]

Compound **12** inhibits FabZ with a K_i value of 11.5 ± 2.8 μM. The K_i value for FabZ inhibition by **37** was determined to be 2.8 ± 1.5 μM (Table 3).

Inhibition of FabI by 12 and 37. The classic double-reciprocal plots to distinguish the inhibitor type of FabI inhibition by **12** and **37** failed to give clear results as expected for tight-binding inhibitors.[22] We observed a strong dependence of the IC_{50} values on the enzyme concentration, that is, the IC_{50} value increases with increasing enzyme concentration at a fixed substrate concentration (see insets in Figure 4A and B). The reversibility of FabI inhibition by **12** and **37** was shown by diluting the inhibited enzyme. In case of an irreversible inhibitor, the enzyme would have remained inactive, which was not observed (data not shown). On the basis of these results, which pointed to the tight-binding inhibition mechanism, we used the tight-binding model to obtain inhibition constants.[22] The binding mechanism of **12** and **37** to FabI was determined by measuring the IC_{50} values at a fixed enzyme concentration while varying the substrate concentration (see the Experimental Section for details). For both **12** (Figure 4A) and **37** (Figure 4B), the IC_{50} values remain constant and independent from the applied substrate or cofactor concentration, suggesting that they are noncompetitive tight-binding inhibitors with $\alpha = 1$.[22] The K_i values were determined by fitting the data obtained from plots of the fractional velocity as a function of inhibitor concentration

Table 3. Kinetic Analysis of **12** and **37** Inhibition on *P. falciparum* FabG, FabZ, and FabI

	inhibitors[a]	
enzyme	**12**	**37**
FabG		
substrate varied[b]	acetoacetyl-CoA	acetoacetyl-CoA
inhibition type	noncompetitive	noncompetitive
replots	linear	linear
K_i (μM)	1.8 ± 0.8	6.4 ± 0.8
α	0.4 ± 0.2	0.5 ± 0.2
substrate varied[c]	NADPH	NADPH
inhibition type	noncompetitive	noncompetitive
replots	linear	linear
K_i (μM)	0.8 ± 0.1	2.8 ± 0.1
α	1.4 ± 0.1	0.8 ± 0.1
FabZ		
inhibition type	competitive	competitive
replots	linear	linear
K_i (μM)	11.5 ± 2.8	2.8 ± 1.5
FabI		
inhibition type	noncompetitive tight binding	noncompetitive tight binding
K_i (μM)	2.1 ± 0.5	0.04 ± 0.01
α[d]	1	1

[a] Reported values represent the mean of at least two experiments. [b] The substrate acetoacetyl-CoA was varied while the cofactor NADPH was kept at a fixed saturating concentration. [c] The cofactor was varied while the substrate remained fixed at saturating conditions. See the Experimental Section for details. [d] The factor α reflects the effect of the inhibitor on the affinity of the enzyme for its substrate and the effect of the substrate on the affinity of the enzyme for the inhibitor. For noncompetitive tight-binding mode, $\alpha = 1$.[22]

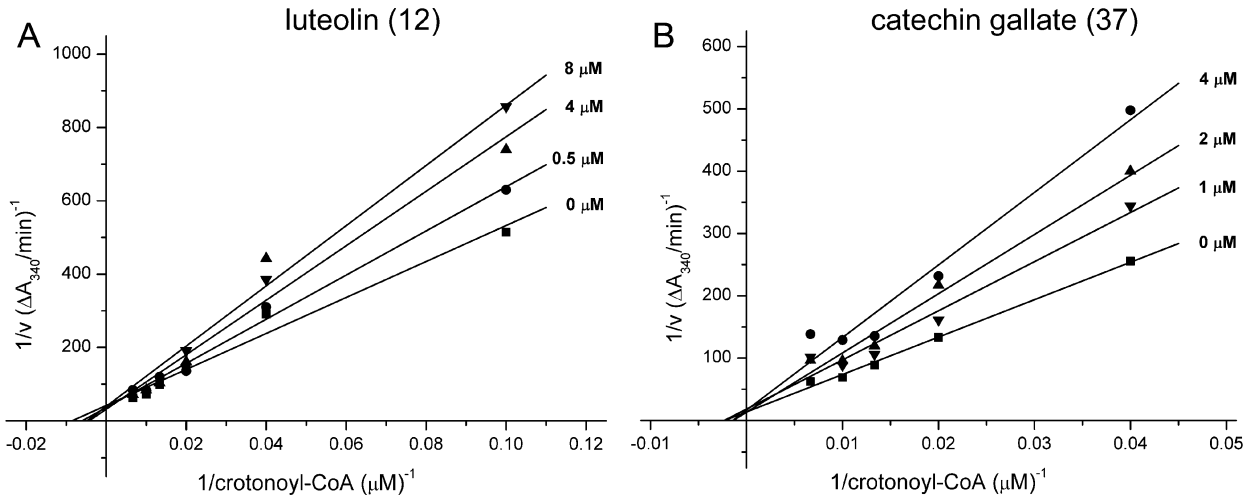


Figure 3. Inhibition mechanism for **12** and **37** on FabZ. Panels A and B show representative double reciprocal plots of $1/v$ vs 1/[substrate] at different inhibitor concentrations. The lines intercepted on the $1/v$ axis, indicating that both **12** (panel A) and **37** (panel B) are competitive inhibitors for the substrate crotonoyl-CoA.

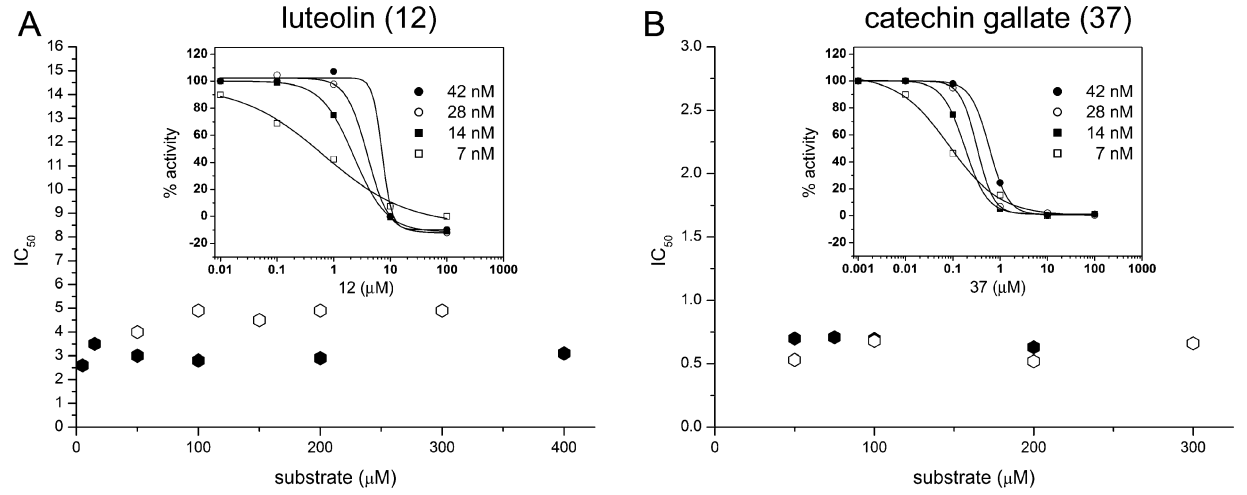


Figure 4. Inhibition mechanism for **12** and **37** on FabI. The insets in panels A and B show the IC_{50} protein concentration dependence for **12** and **37**. The enzyme concentrations used (7−42 nM) are indicated in the insets (●, ○, ■, and □). The increasing IC_{50} values when measured at several increasing enzyme concentration is indicative of a tight-binding mechanism.[22] To distinguish the inhibitor type, the IC_{50} values were determined by varying the substrate crotonoyl-CoA (⬢) or the cofactor NADH (⬡) concentrations while keeping the enzyme concentration fixed at 42 nM (see the Experimental Section for details). The IC_{50} values for both **12** and **37** remained constant with respect to substrate and cofactor, suggesting that they are noncompetitive tight-binding inhibitors with $\alpha = 1$.[22]

to the Morrison equation.[23] The K_i values obtained are 2.1 ± 0.5 and 0.04 ± 0.01 μM for **12** and **37**, respectively (Table 3).

In Vitro Whole-Cell Assay against *P. falciparum* NF54 and K1. Antimalarial activity of the polyphenols toward the chloroquine-sensitive strain NF54 is shown in Table 1 and Table 2. A number of compounds (**10**−**14**) show activity against *P. falciparum* and are also potent inhibitors of FabG, FabZ, and FabI, exhibiting IC_{50} values in the range of 8−30 μM. These values are similar to the ones observed for the inhibition of the isolated enzymes (Table 1). Promising inhibition activity against parasite cells and either one or two FAS-II enzymes are also observed for **4**, **10**, and **18**. Among the catechins tested, only **37** strongly interferes with parasite growth and demonstrates enzyme inhibition at the same time. Despite their powerful action toward the three enzymes, the antimalarial potency of the remaining C-3 gallate esters **32**, **33**, and **38** is only moderate. The most active and promising compounds (**12**−**16**, **32**, **33**, **37**, and **38**) were also tested against the chloroquine-resistant K1 strain (Table 1 and Table 2). Flavonoids **12**−**14** exhibit activity against strain K1 similar to that observed for NF54, whereas compounds **15** and **16** and catechins **32**, **33**, **37**, and **38** show increased antiplasmodial activity toward chloroquine-resistant *P. falciparum*. Overall, the best activity is exerted by compound **37** (IC_{50} 0.4 μM)

In general, all compounds tested have at least modest antiplasmodial activity that is correlated to FabG, FabZ, and FabI inhibition. However, 3-hydroxyflavone (**2**), ladanein (**19**), and 5,4′-dihydroxy-6,7-dimethoxyflavanone (**23**) do not inhibit any of the three target enzymes but are highly active in the in vitro assay. This suggests that their corresponding targets are different from the fatty acid biosynthesis pathway of *P. falciparum*.

Discussion

On the basis of the screening results of our natural product library,[21] we identified the aglycone (**12**) of luteolin-7-*O*-glucoside as a promising new chemical entity with good plasmodial FabI inhibition activity. This study encouraged us to investigate the potential of various flavonoids to inhibit FabI, and we extended our search to FabG and FabZ. Kinetic analyses were carried out to provide insight into the mechanism by which the three enzymes are inhibited by polyphenols.

Inhibition Mechanisms for FabG, FabZ, and FabI. Both **12** and **37** behave in a noncompetitive manner against FabG with respect to the substrate and the cofactor. Thus, **12** and **37** display affinity for both the free enzyme and the binary enzyme−substrate complex. As shown in Figure 2 (panels C and D), the lines intersect below the *x* and *y* axis at negative values of 1/[substrate] and $1/v$ for **37** inhibition. This results in a value of $\alpha < 1$, suggesting that the binding of the inhibitor increases the affinity of the substrate and cofactor to the enzyme and vice versa.[22] According to these results, it can be assumed that **37** binds to a site distinct from the substrate and cofactor binding sites, thereby inducing a conformational change in the enzyme structure that allows substrate binding at increased affinity. Similarly, if the substrates have bound first, the same change in conformation leads to improved inhibitor-binding affinity. The same holds for **12** (Figure 2A) with respect to acetoactyl-CoA. With respect to NADPH (Figure 2B), the lines intersect above the *x* and *y* axis at positive values of 1/[substrate] and $1/v$. The value of $\alpha > 1$ represents the case where the binding of the inhibitor negatively influences the affinity for the cofactor and vice versa. Such conformational adaptations are well known for binding events, and with respect to the FabG homologue in *E. coli*, it has been observed that the biochemical properties and the catalytic mechanism of FabG are directly controlled by major conformational changes in the protein.[24,25]

In contrast to FabG, **12** and **37** act as competitive inhibitors of FabZ. This indicates that the inhibitors and the substrate bind to the same site in the free enzyme in a mutually exclusive fashion.

The situation for the activity og **12** and **37** against FabI is more complex. The classical double-reciprocal plots did not allow the determination of the inhibition type. Because the system met several of the criteria for a tight-binding model, the data was treated accordingly. Both inhibitors appear to act as noncompetitive tight-binding inhibitors with $\alpha = 1$, which means that **12** and **37** bind to a site that is distinct to the substrate and cofactor binding site. The inhibitor and the substrates do not influence their binding affinities to the enzyme, as can be deduced from $\alpha = 1$. Interestingly, a recent report[20] identified **33** as an efficient inhibitor of *E. coli* FabI, and the authors observed competitive behavior with respect to the cofactor NADH. Although **37** and **33** are different molecules, with **33** bearing an additional hydroxyl group at the 5′ position of ring B and exhibiting 2R instead of the 2S configuration of **37**, it is surprising to observe this different binding mode. The results of preliminary kinetic experiments using **33** were very similar to the ones obtained with **37** (data not shown). The mechanistic reason for this difference is unclear. However, although the sequence identity of 21% is rather low, the overall structural similarity between *P. falciparum* and *E. coli* FabI is high with an RMS of 0.78 Å for Cα atoms.[2] A close inspection of the NADH binding sites in the crystal structures of *P. falciparum* FabI and *E. coli* FabI with the bound cofactor (pdb entries 1VRW [2] and 1DFI,[26] respectively) reveals a series of differences with respect to cofactor interaction with the neighboring amino acids. In general, the cofactor binding site is deeper and formed by more amino acids in *P. falciparum* FabI than in *E. coli* FabI. Some of the important differences in the NADH binding pocket of *P. falciparum* FabI compared to *E. coli* FabI are mediated through residues Tyr111 (corresponding residue in *E. coli* FabI: Ile20), Trp131 (Qln40), Ala169 (Val65), Ala217 (Gly93), Asn218 (Phe94), Lys285 (Lys163), Leu315 (Ile192), and Ser317 (Thr194). An analysis of the NADH−protein interactions shows that the NADH binding sites in *E. coli* FabI and *P. falciparum* FabI are significantly different. Hydrophobic interactions as well as the number of hydrogen bonds are increased in the parasite enzyme. Considering these structural differences, it is likely that **37** and **33** inhibit *P. falciparum* FabI with a different mechanism than the one identified for *E. coli* FabI.

Structure−Activity Relationship for FabG, FabZ, and FabI Inhibition. To gain deeper insight into the structural characteristics necessary or important for inhibitor binding, a series of natural polyphenols were tested against all three enzymes. The simple flavones and flavonols, which have no hydroxy groups on ring B and one or two hydroxy groups on the A/C ring system (Table 1, compounds **1**−**9**), have little or no effect on the enzymes tested. A single hydroxylation at ring B in combination with a flavone structure (as found in **10**) has no significant activity, but the combination of a *p*-hydroxyphenyl side chain with a flavonol structure as found in kaempferol (**11**) improves inhibition. At the same time, however, the selectivity seems to be reduced because the compound is active against both FabI and FabG. The same trend can be observed for the compounds carrying an even more complex hydroxylation pattern. If phenyl ring B is hydroxylated at two or three positions, regardless of an additional hydroxy group at C-3 (**12**−**16**), the polyphenols become very potent inhibitors of all three enzymes. The combination of the presence of a pyrogallol (3′,4′,5′-trihydroxyphenyl) moiety in ring B and of a flavonol structure (as found in **16**) increases the selectivity against the FabI enzyme. In contrast, the methylation of any of the phenolic hydroxy groups leads to a total loss of inhibition capacity against all enzymes in almost all cases (**19**−**25**), **18** being the only exception. The isoflavonoids examined in this study (**26**−**28**) show a different trend. With phenyl ring B now attached to the C-3 position of ring C, the compounds seem to target only FabZ while being inactive against FabG and FabI.

In summary, the hydroxylation pattern within a flavonoid structure plays an important role in the recognition process. Highly hydroxylated compounds show very good enzyme inhibition with minimal selectivity. This observation is very intriguing because inhibitors with triple activity are rarely described, and such compounds would offer a great advantage over entities that act only against one target. It is very unlikely that the malaria parasite would be able to develop resistance against such triple action inhibitors by means of active site mutations.

Another group of polyphenols with significant activity include the catechins with a flavan-3-ol structure. The lack of activity of the catechins bearing no galloyl group at C-3 (**29**−**31** and **34**−**36**) indicates that gallate substitution is essential for inhibition activity (Table 2). Whereas all catechins with a free hydroxy group at position C-3 are inactive, all C-3 galloyl ester derivatives (**32**, **33**, **37**, and **38**) show excellent inhibition potential against all three enzymes. Interestingly, gallic acid and other polyhydroxybenzoic acids alone are inactive (data not shown). As deduced from the almost identical behavior of **37** (2S configuration) and **32** (2R configuration) derivatives, the stereochemistry at C-2 does not play an important role in the inhibition of FabG, FabZ, or FabI. Unfortunately, the corresponding enantiomers of **37** and **32**, that is, (+)-catechin gallate (2R, 3S) and (+)-epicatechin gallate (2S, 3S), are not commercially available and could not be tested to further investigate the importance of the stereochemistry at C-3. Nevertheless, our data are in very good agreement with the recently published results regarding FabG and FabI of *E. coli*.[20] It also seems that the influence of the hydroxylation pattern of ring B is low because the enzyme inhibitory activity of catechins with a

catechol (3′,4′-dihydroxyphenyl) or a pyrogallol (3′,4′,5′-trihydroxyphenyl) motive on ring B are comparable.

An additional aspect arises when **10** is compared to its flavanon derivative naringenin (**17**). The loss of the double bond between C-2 and C-3 abolishes the activity of compound **10**, indicating the importance of the planarity of flavonoids with respect to activity. Indeed, all active flavonoids comprise a planar structure. A similar observation can be deduced for the pair **23** and **24**. The catechin compounds without a galloyl group at C-3 (**29**−**31** and **34**−**36**) fit well with the planarity concept. Because of the missing double bond, they apparently deviate from the planar conformation suitable for binding to the targets. In contrast, all catechins carrying a galloyl group at C-3 (**32**, **33**, **37**, and **38**) are very potent inhibitors. Apparently, the additional galloyl moiety represents a key feature to overcome the need for planarity, most probably by inducing a different binding mode than that of the flavonoids. This is further supported by the fact that **37** binding to FabG with respect to NADPH exhibits a value of $\alpha < 1$, indicating a different binding mode than the corresponding interaction with **12**, which shows $\alpha > 1$ (Figure 2B and D; Table 3).

In Vitro Efficacy of Polyphenol Inhibitors. All polyphenols outlined in Tables 1 and 2 were examined for in vitro antiplasmodial activity against the chloroquine-sensitive *P. falciparum* strain NF54. In addition, a selection of the most promising and active compounds was also tested against the chloroquine-resistant parasite strain K1. Although a number of compounds show good in vitro activity that correlates well with FabG, FabZ, and FabI inhibition (see the Results Section), some polyphenols do not inhibit any of the three targets but still possess high in vitro antimalarial activity. These results suggest that polyphenols also act on targets in the parasite cell other than the FAS-II pathway. Indeed, recent investigations on *E. coli* fatty acid biosynthesis failed to directly correlate FAS-II inhibition with antibacterial activity mediated by polyphenols.[20] This is in agreement with the ability of flavonoids to interfere with many essential biochemical pathways.[8−10] It was also shown that a number of flavonoids as well as epicatechins inhibit the human type-I fatty acid biosynthesis system. [17,27−29] In light of these findings, it must be emphasized that although the inhibition results on the isolated enzymes and their activity in the whole-cell assay are very promising, further development of flavonoid-type lead compounds will require more detailed in vitro and in vivo studies to evaluate *P. falciparum* FAS-II as the main target.

Future Aspects of the Inhibitors. With respect to the potential use of polyphenols as antiplasmodial agents, their success will strongly depend on attaining relevant concentrations in tissues, that is, on absorption, distribution, metabolism, and excretion of the compounds. In general, flavonoids display very low toxicity to humans and can accumulate to concentrations up to 8 μM in human plasma while exhibiting half-lives ranging from 1 to 28 h.[30−32] Considering these facts, the compounds described in this study represent an interesting class of secondary plant metabolites with potent activity against isolated enzymes of the *P. falciparum* FAS-II pathway. They appear to constitute interesting chemical scaffolds for further development to improve the pharmacokinetic profiles as well as selectivity against protozoa. The results from the kinetic analysis indicate that polyphenols bind to FabG and FabI at a site distinct from the substrate and cofactor binding sites (noncompetitive inhibition). The dehydratase inhibition is competitive, and thus, the flavonoids interact with the substrate site. A very interesting observation is the fact that individual compounds are capable of functioning as inhibitors of three enzymes in the same pathway. This opens the field for the development of a new type of drug with some remarkable features, such as increased efficacy (one drug for three targets) and decreased risk of developing resistance.

Experimental Section

Materials. Aldrich (Buchs, Switzerland) supplied flavone (**1**), 6-hydroxyflavone (**4**), 3,6-dihydroxyflavone (**6**), and 3,7-dihydroxyflavone (**7**), and Sigma (Buchs, Switzerland) supplied 5-hydroxyflavone (**3**), 7-hydroxyflavone (**5**), and all catechin type compounds (**29**−**38**). Chrysin (**8**), apigenin (**10**), luteolin (**12**), galangin (**9**), kaempferol (**11**), fisetin (**14**), quercetin (**13**), myricetin (**16**), and morin (**15**) were purchased from Fluka (Buchs, Switzerland). Roth (Karlsruhe, Germany) supplied quercetin-3,7,3′,4′-tetramethyl ether (**21**), naringenin (**17**), 5,7-dimethoxy-8-methylflavanone (**25**), and 5,4′-dihydroxy-6,7-dimethoxyflavanone (**23**). Compound **2** (3-hydroxyflavone) was purchased from ABCR (Karlsruhe, Germany), whereas **6**, **7**, **18**, **22** and **26**−**28** were purchased from Extrasynthese (Genay, France). All compounds were of the highest purity available (generally 95−99%). Ladanein (**19**), cirsimaritin (**24**), and genkwanin (**20**) were previously isolated from Lamiaceae plants and were of >95% purity.[33−36] Roche Diagnostics (Rotkreuz, Switzerland) supplied NADH and NADPH; a terrific broth medium was obtained from Difco (Kansas City, MO). *E. coli* BL21(DE3) CodonPlus-RIL cells were purchased from Stratagene (La Jolla, CA), and the HiTrap metal chelating and Superdex75/200 gel filtration columns were obtained from GE Healthcare (Otelfingen, Switzerland). All pET vectors used in this study were purchased from Novagen (Darmstadt, Germany). The spectrophotometric measurements were carried out on a Cary 50 conc Varian Spectrophotometer (Zug, Switzerland). All polyphenols tested in this study were dissolved in DMSO to give a 100 mM stock solution.

Expression and Purification of FabG. The protein was cloned using a strategy similar to that described for FabI [2]. Briefly, the *pffabG* sequence *N*-terminally truncated by 162 base pairs was PCR-amplified from a *P. falciparum* (3D7 strain) gametocyte cDNA pSPORT plasmid library (kindly provided by Dr. Thomas Templeton, Weill Medical College, Cornell University, New York) and after restriction digestion, ligated into the pET30b expression vector. Correct clones were identified and verified by restriction digestion, PCR, and automated sequencing. The expression clone expressed the enzyme without the putative *N*-terminal signal and translocation sequence, that is, amino acids 1−54. Thus, FabG, entailing residues 55−304, was expressed at 37 °C using BL21(DE3) CodonPlus-RIL cells. Purification of the protein was performed as described for FabI [2], except that a linear imidazole gradient was applied, that is, the imidazole concentration was raised from 20 to 500 mM in 25 min at a flow rate of 2 mL/min. This clone offered great advantage over recently described methods because it could express high amounts of soluble and active FabG without the need for refolding procedures.[37,38]

Expression and Purification of FabZ. The protein was cloned, expressed, and purified as described.[39] Correct clones were identified and verified by restriction digestion, PCR, and automated sequencing. FabZ was expressed without the putative *N*-terminal signal- and translocation-sequences, that is, amino acids 1−80. FabZ, consisting of residues 81−230, was expressed at <15 °C for 24 h using BL21(DE3) CodonPlus-RIL cells. Cell pellets were disrupted using a French press, and the enzyme was isolated by metal-chelate-affinity chromatography. All fractions containing the enzyme were collected, concentrated, and applied to a Superdex75 gel filtration column equilibrated with 20 mM Hepes at pH 7.4 and 100 mM NaCl.

Expression and Purification of FabI. The protein was cloned, expressed, and purified as described [2]. Correct clones were identified and verified by restriction digestion, PCR, and automated sequencing. The resulting expression clone was designed to express FabI without the *N*-terminal signal- and translocation-sequence, that is,

amino acids 1−77. Subsequently, mature length FabI, including residues 78−432, was expressed for 5 h at 37 °C in BL21(DE3) CodonPlus-RIL cells. The cell pellets were suspended and disrupted using a French press, and the protein was isolated by means of metal-chelate-affinity chromatography. The protein was concentrated and applied to a Superdex200 gel filtration column equilibrated with 20 mM Tris at pH 7.5 and 150 mM NaCl.

FabG Assay. All measurements were performed at 25 °C in 20 mM Hepes at pH 7.0, 35 mM NaCl, and 1% DMSO followed by the oxidation of NADPH to $NADP^+$ for 1 min at 340 nm using acetoacetyl-CoA as substrate. The IC_{50} values were determined in a final volume of 1000 μL in the presence of 1 μg (36 nM) of enzyme and 100 μM NADPH while varying the compound concentration. The reaction was started by the addition of 50 μM acetoacetyl-CoA. The values for IC_{50} were estimated from graphically plotted dose−response curves. The type of inhibition was determined with respect to the substrate acetoacetyl-CoA and the cofactor NADPH in consideration of Michaelis−Menten steady-state conditions. To investigate the inhibition mechanism with respect to acetoacetyl-CoA, FabG (0.5 μg = 45 nM) was incubated for 10 min at room temperature with a fixed and saturating NADPH concentration (300 μM) and different inhibitor concentrations (0−4 μM) in a total volume of 400 μL. The reaction was initiated by the addition of acetoacetyl-CoA (10−600 μM). The inhibition mechanism with respect to NADPH was determined in a similar way. To this end, FabG was incubated with varying NADPH concentrations (10−300 μM) and different inhibitor concentrations (0−4 μM). The reaction was initiated by the addition of 600 μM acetoacetyl-CoA. The K_i values were obtained from Dixon and secondary plots. The reported values represent the mean of at least two independent experiments.

FabZ Assay. The standard reaction mixture for the FabZ assay consisted of 20 μg (2.9 μM) of FabZ in 20 mM Hepes at pH 7.4, 150 mM NaCl, and 1% DMSO in a total volume of 400 μL. The decrease in absorption at 263 nm using crotonoyl-CoA as substrate was continuously monitored for 5 min at 25 °C. For IC_{50} determination, FabZ was incubated with varying concentrations of the compounds. The reaction was started with 100 μM crotonoyl-CoA, and the IC_{50} values were estimated from graphically plotted dose−response curves. The inhibition mechanism was determined in the presence of various inhibitor concentrations (0−8 μM) and in consideration of the steady-state conditions. After 10 min of incubation time, the reaction was started by the addition of crotonoyl-CoA (10−150 μM). The K_i values were obtained from Dixon and secondary plots. The mean of at least two independent experiments are reported.

FabI Assay. FabI was assayed at 25 °C in 20 mM Hepes at pH 7.4, 150 mM NaCl, and 1% DMSO, monitoring, for 1 min, the decrease in absorbance at 340 nm due to the consumption of cofactor NADH while using crotonoyl-CoA as a substrate. The IC_{50} values for the compounds were determined essentially as described for FabG but using 1 μg (22 nM) of enzyme, 50 μM crotonoyl-CoA as substrate, and 100 μM NADH as cofactor. The IC_{50} protein concentration dependence was measured under the same conditions using FabI concentrations in the range of 7−42 nM. The mechanism of inhibition was determined, considering the Michaelis−Menten conditions, by analyzing the relationship of IC_{50} values versus substrate and cofactor concentrations as described in the literature.[40] For the substrate, FabI (0.75 μg = 42 nM) was incubated for 10 min at room temperature with varying concentrations of the inhibitor (0−20 μM) in the presence of 300 μM NADH in a total volume of 400 μL before the reaction was started with crotonoyl-CoA. The IC_{50} values were determined for several crotonoyl-CoA concentrations (10−400 μM). The IC_{50} values with respect to the cofactor were determined in a similar way. After incubating FabI with the inhibitor (0−20 μM) in the presence of varying NADH concentrations (50−300 μM), the reaction was started with 600 μM crotonoyl-CoA. For the determination of K_i values, the fractional velocity as a function of inhibitor concentration was measured at fixed enzyme (42 nM), substrate (400 μM), and cofactor (300 μM) concentrations. The K_i values were generated by fitting the obtained data to the Morrison equation assuming noncompetitive inhibition.[23] The results represent the mean of two independent measurements.

In Vitro *P. falciparum* Assay. Antiplasmodial activity was determined using the chloroquine-sensitive *P. falciparum* strain NF54 and the chloroquine-resistant strain K1. A modified [^{3}H]-hypoxanthine incorporation assay was applied as described before.[41,42] Briefly, infected human red blood cells cultivated in an RPMI 1640 medium with 5% Albumax II were exposed to serial drug dilutions in microtiter plates. After 48 h of incubation at 37 °C in a reduced oxygen atmosphere, 0.5 μCi [^{3}H]-hypoxanthine was added to each well. Viability was determined by measuring [^{3}H]-hypoxanthine incorporation by means of liquid scintillation counting after further 24 h of incubation time. The results were recorded as counts/min/well at each drug concentration and expressed as a percentage of the untreated controls. The IC_{50} values were calculated from the sigmoidal inhibition curves. The reported results represent the mean of duplicate assays. Artemisinin served as the positive control.

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References

(1) Hay, S. I.; Guerra, C. A.; Tatem, A. J.; Noor, A. M.; Snow, R. W. The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect. Dis.* **2004**, *4*, 327−336.
(2) Perozzo, R.; Kuo, M.; Sidhu, A. b. S.; Valiyaveettil, J. T.; Bittman, R.; et al. Structural elucidation of the specificity of the antibacterial agent triclosan for malarial enoyl acyl carrier protein reductase. *J. Biol. Chem.* **2002**, *277*, 13106−13114.
(3) Surolia, N.; Surolia, A. Triclosan offers protection against blood stages of malaria by inhibiting enoyl-ACP reductase of *Plasmodium falciparum*. *Nat. Med.* **2001**, *7*, 167−173.
(4) Gornicki, P. Apicoplast fatty acid biosynthesis as a target for medical intervention in apicomplexan parasites. *Int. J. Parasitol.* **2003**, *33*, 885−896.
(5) Roberts, C. W.; McLeod, R.; Rice, D. W.; Ginger, M.; Chance, M. L.; et al. Fatty acid and sterol metabolism: potential antimicrobial targets in apicomplexan and trypanosomatid parasitic protozoa. *Mol. Biochem. Parasitol.* **2003**, *126*, 129−142.
(6) Waller, R. F.; Ralph, S. A.; Reed, M. B.; Su, V.; Douglas, J. D.; et al. A type II pathway for fatty acid biosynthesis presents drug targets in *Plasmodium falciparum*. *Antimicrob. Agents Chemother.* **2003**, *47*, 297−301.
(7) Koes, R. E.; Quattrocchio, F.; Mol, J. N. M. The flavonoid biosynthetic pathway in plants: function and evolution. *BioEssays* **1994**, *16*, 123−132.
(8) Harborne, J. B.; Williams, C. A. Advances in flavonoid research since 1992. *Phytochemistry* **2000**, *55*, 481−504.
(9) Robak, J.; Gryglewski, R. J. Bioactivity of flavonoids. *Pol. J. Pharmacol.* **1996**, *48*, 555−564.
(10) Williams, C. A.; Grayer, R. J. Anthocyanins and other flavonoids. *Nat. Prod. Rep.* **2004**, *21*, 539−573.
(11) Hollman, P. C.; Hertog, M. G.; Katan, M. B. Role of dietary flavonoids in protection against cancer and coronary heart disease. *Biochem. Soc. Trans.* **1996**, *24*, 785−789.
(12) Knekt, P.; Jarvinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary mortality in Finland: a cohort study. *Br. Med. J.* **1996**, *312*, 478−481.
(13) Rice-Evans, C. A.; Miller, N. J.; Bolwell, P. G.; Bramley, P. M.; Pridham, J. B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Res.* **1995**, *22*, 375−383.
(14) Aviram, M.; Fuhrman, B. Polyphenolic flavonoids inhibit macrophage-mediated oxidation of LDL and attenuate atherogenesis. *Atherosclerosis* **1998**, *137*, S45−50.
(15) Fitzpatrick, D. F.; Bing, B.; Maggi, D. A.; Fleming, R. C.; O'Malley, R. M. Vasodilating procyanidins derived from grape seeds. *Ann. N.Y. Acad. Sci.* **2002**, *957*, 78−89.
(16) Herrera, M. D.; Zarzuelo, A.; Jimenez, J.; Marhuenda, E.; Duarte, J. Effects of flavonoids on rat aortic smooth muscle contractility: structure-activity relationships. *Gen. Pharmacol.* **1996**, *27*, 273−277.
(17) Li, B. H.; Tian, W. X. Inhibitory effects of flavonoids on animal fatty acid synthase. *J. Biochem. (Tokyo)* **2004**, *135*, 85−91.

(18) Li, X. C.; Joshi, A. S.; ElSohly, H. N.; Khan, S. I.; Jacob, M. R.; et al. Fatty acid synthase inhibitors from plants: isolation, structure elucidation, and SAR studies. *J. Nat. Prod.* **2002**, *65*, 1909−1914.
(19) Wang, X.; Song, K. S.; Guo, Q. X.; Tian, W. X. The galloyl moiety of green tea catechins is the critical structural feature to inhibit fatty-acid synthase. *Biochem. Pharmacol.* **2003**, *66*, 2039−2047.
(20) Zhang, Y. M.; Rock, C. O. Evaluation of epigallocatechin gallate and related plant polyphenols as inhibitors of the FabG and FabI reductases of bacterial type II fatty-acid synthase. *J. Biol. Chem.* **2004**, *279*, 30994−31001.
(21) Kirmizibekmez, H.; Calis, I.; Perozzo, R.; Brun, R.; Donmez, A. A.; et al. Inhibiting activities of the secondary metabolites of *Phlomis brunneogaleata* against parasitic protozoa and plasmodial enoyl-ACP reductase, a crucial enzyme in fatty acid biosynthesis. *Planta Med.* **2004**, *70*, 711−717.
(22) Copeland, R. A. *ENZYMES: A Practical Introduction to Structure, Mechanism, and Data Analysis*, 2nd ed.; Wiley-VCH: New York, 2000; p 397.
(23) Morrison, J. F. Kinetics of the reversible inhibition of enzyme-catalysed reactions by tight-binding inhibitors. *Biochim. Biophys. Acta* **1969**, *185*, 269−286.
(24) Price, A. C.; Zhang, Y. M.; Rock, C. O.; White, S. W. Structure of beta-ketoacyl-[acyl carrier protein] reductase from *Escherichia coli*: negative cooperativity and its structural basis. *Biochemistry* **2001**, *40*, 12772−12781.
(25) Price, A. C.; Zhang, Y. M.; Rock, C. O.; White, S. W. Cofactor-induced conformational rearrangements establish a catalytically competent active site and a proton relay conduit in FabG. *Structure* **2004**, *12*, 417−428.
(26) Baldock, C.; Rafferty, J. B.; Stuitje, A. R.; Slabas, A. R.; Rice, D. W. The X-ray structure of *Escherichia coli* enoyl reductase with bound NAD+ at 2.1 A resolution. *J. Mol. Biol.* **1998**, *284*, 1529−1546.
(27) Wang, X.; Tian, W. Green tea epigallocatechin gallate: a natural inhibitor of fatty-acid synthase. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 1200−1206.
(28) Brusselmans, K.; De Schrijver, E.; Heyns, W.; Verhoeven, G.; Swinnen, J. V. Epigallocatechin-3-gallate is a potent natural inhibitor of fatty acid synthase in intact cells and selectively induces apoptosis in prostate cancer cells. *Int. J. Cancer* **2003**, *106*, 856−862.
(29) Brusselmans, K.; Vrolix, R.; Verhoeven, G.; Swinnen, J. V. Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthase activity. *J. Biol. Chem.* **2005**, *280*, 5636−5645.
(30) Williamson, G.; Manach, C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am. J. Clin. Nutr.* **2005**, *81*, 243S-255S.
(31) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81*, 230S-242S.
(32) Walle, T. Absorption and metabolism of flavonoids. *Free Radical Bio. Med.* **2004**, *36*, 829−837.
(33) Tasdemir, D.; Calis, I.; Sticher, O. Labdane diterpenes from *Leonurus persicus*. *Phytochemistry* **1998**, *49*, 137−143.
(34) Liu, G.; Rüedi, P. Phyllocladanes (13β-kauranes) from *Plectranthus ambiguus*. *Phytochemistry* **1996**, *41*, 1563−1568.
(35) Juch, M.; Rüedi, P. Isolation, structure, and biological activities of long-chain catechols of *Plectranthus sylvestris* (*Labiatae*). *Helv. Chim. Acta* **1997**, *80*, 436−448.
(36) Bürgi, C.; Rüedi, P. Isolierung und struktur von langkettigen alkylphenolen und -pyrocatecholen aus *Plectranthus albidus* (*Labiatae*) (Isolation and structure of long-chain alkylphenols and catechols from *Plectranthus albidus* (*Labiatae*)). *Helv. Chim. Acta* **1993**, *76*, 1890−1900.
(37) Pillai, S.; Rajagopal, C.; Kapoor, M.; Kumar, G.; Gupta, A.; et al. Functional characterization of beta-ketoacyl-ACP reductase (FabG) from *Plasmodium falciparum*. *Biochem. Biophys. Res. Commun.* **2003**, *303*, 387−392.
(38) Karmodiya, K.; Srivastav, R. K.; Surolia, N. Production and purification of refolded recombinant *Plasmodium falciparum* beta-ketoacyl-ACP reductase from inclusion bodies. *Protein Expression Purif.* **2005**, *42*, 131−136.
(39) Kostrewa, D.; Winkler, F. K.; Folkers, G.; Scapozza, L.; Perozzo, R. The crystal structure of PfFabZ, the unique beta-hydroxyacyl-ACP dehydratase involved in fatty acid biosynthesis of *Plasmodium falciparum*. *Protein Sci.* **2005**, *14*, 1570−1580.
(40) Williams, J. W.; Morrison, J. F. The kinetics of reversible tight-binding inhibition. *Methods Enzymol.* **1979**, *63*, 437−467.
(41) Sperandeo, N. R.; Brun, R. Synthesis and biological evaluation of pyrazolylnaphthoquinones as new potential antiprotozoal and cytotoxic agents. *ChemBioChem* **2003**, *4*, 69−72.
(42) Matile, H.; Pink, J. R. L. *Plasmodium falciparum* malaria parasite cultures and their use in immunology. *Immunological Methods*; Academic Press: San Diego, CA, 1990; pp 221−234.
(43) Wallace, A. C.; Laskowski, R. A.; Thornton, J. M. LIGPLOT: A program to generate schematic diagrams of protein−ligand interactions. *Protein Eng.* **1995**, *8*, 127−134.

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