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Synthesis and antimalarial evaluation of a series of piperazinyl flavones

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Abstract—A series of 27 flavonoid derivatives containing a piperazinyl chain have been synthesized and tested for their antiplasmodial activity. Diverse substitution patterns on piperazinyl and flavone moieties were examined and found to affect the activity differently. The most active compounds, which have a 2,3,4-trimethoxybenzylpiperazinyl chain attached to the flavone at the 7-phenol group, showed in vitro activity against chloroquine-sensitive (Thai) and -resistant (FcB1, K1) *Plasmodium falciparum* strains in the micromolar to submicromolar range. One of them was active when given orally in a *Plasmodium yoelii nigeriensis* infected mouse model.

The development of multidrug-resistant *Plasmodium* strains represents a major problem in the treatment of malaria. In a previous publication, we synthesized a series of flavonoid derivatives containing a *N*-benzylpiperazine chain, and reported on their ability to potentiate doxorubicin cytotoxicity on resistant K562/doxorubicin cells through multidrug resistance (MDR)-modulating activity.[1] More recently, some synthesized chromone derivatives with a *N*-substituted piperazine fragment proved also to be powerful reversal agents of MDR.[2,3] As verapamil, a well-known MDR modulator, was shown to partially reverse *Plasmodium falciparum* chloroquine resistance,[4] we decided to explore the chloroquine-potentiating effect of this series of synthetic flavonoids on *P. falciparum*. No synergistic effect was noted, but an intrinsic antiplasmodial activity[5] was observed with most of studied compounds, especially flavones **1** and **8** (IC_{50} 0.5 and 0.18 μM) (Table 1). Some clear structure–activity relationships emerged from this study: a polymethoxylation pattern of the benzylpiperazinyl chain and its fixation at the 7-phenol group, and an increased lipophilic flavone moiety were favorable to the activity. It is noteworthy that both parts (flavone and benzylpiperazinyl) of these hybrid structures were only weakly active on *P. falciparum* by themselves: IC_{50} of diosmetin (**11**) and its diether **12** were, respectively, 36 and 70 μM, in the range of literature data for flavones,[6,7] while 2,3,4-trimethoxybenzylpiperazine (**13**) and its *N*-acetyl derivative were almost inactive (IC_{50} 100 μM) (data not shown). Starting from these promising results, compound **1**, slightly less active than **8** but devoid of cytotoxicity upon the mammalian cells MCR5[8] (Table 1), was selected as a model for pharmacomodulation studies. Therefore, we report in this letter on the synthesis and the antiplasmodial activity of two series of analogs of **1**, different from the model in the piperazinyl chain (compounds **20**–**30**), or in the flavone moiety (compounds **31**–**36**, Figs. 1 and 2).

Keywords: *Plasmodium falciparum*; Antimalarial drugs; Flavonoids; Diosmin; Piperazinyl chain.
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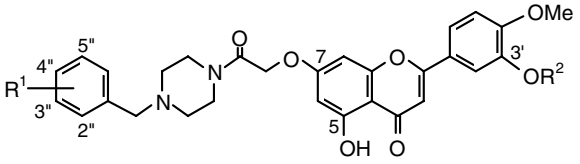
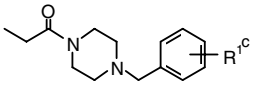
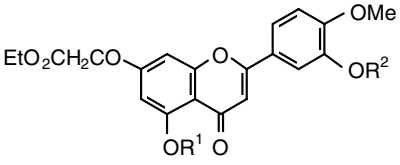
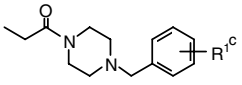
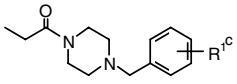
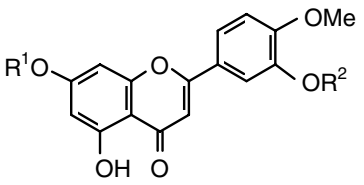
Table 1. Structure and in vitro antiplasmodial activities of previously synthesized compounds **1**–**10**



Compound	R^1	R^2	Activity[a] IC_{50} (μM)
1	2″,3″,4″-$(OMe)_3$	CH_2CO_2Et	0.5[b]
2	2″,3″-$(OMe)_2$	CH_2CO_2Et	2.4
3	3″,4″,5″-$(OMe)_3$	CH_2CO_2Et	4.0
4	3″,4″-$(OMe)_2$	CH_2CO_2Et	4.7
5	4″-Cl	CH_2CO_2Et	6.0
6	H	CH_2CO_2Et	9.5
7	2″,3″,4″-$(OMe)_3$	H	5.3
8	2″,3″,4″-$(OMe)_3$		0.18[d]
			
9	H		6.2
10		CH_2CO_2Et	5.4

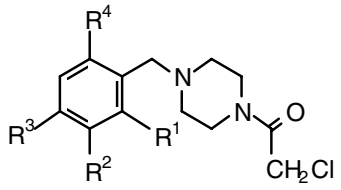
Chloroquine was used as positive control (IC_{50} 0.18 μM).
[a] Antiplasmodial activity was evaluated on the D6 strain of *P. falciparum*.
[b] Cytotoxicity on MRC5 cells: $IC_{50} > 70$ μM.
[c] R^1 = 2″,3″,4″-$(OMe)_3$.
[d] Cytotoxicity on MRC5 cells: $IC_{50} = 1$ μM. Determinations are means of three independent experiments.

Synthesis of analogs of **1** *by modification of the piperazinyl moiety*. The synthetic strategy for the preparation of amides **20**–**29** involved the preparation of substituted *N*-(chloroacetyl)benzylpiperazines **19** and their coupling through the 7-phenol alkylation of **15**, a semi-synthetic flavone readily available from the natural flavonoid diosmin (**14**).[9] *N*-(Chloroacetyl)benzylpiperazines **19a**–**e** were synthesized from the appropriate benzaldehyde according to a previously described method.[1] *N*-(Chloroacetyl)benzylpiperazine **19f** resulted particularly from an initial Mannich reaction between 3-methoxycatechol and ethyl 1-piperazinecarboxylate followed by dialkylation of the catechol system by CH_2Br_2.[10] Lastly, condensation of *tert*-butyl 1-piperazinecarboxylate with chloroacetyl chloride led to the corresponding chloroacetamide. Coupling of all these chloroacetylpiperazines



	R^1	R^2
11	H	H
12	CH_2CO_2Et	CH_2CO_2Et
14	rutinosyl	H
15	H	CH_2CO_2Et
16	H	CH_2CO_2t-But
17	H	*n*-hexyl
18	H	cyclopentyl

Figure 1.



	R^1	R^2	R^3	R^4
19 a	OMe	OMe	OMe	H
19 b	H	H	OMe	H
19 c	OMe	H	OMe	H
19 d	OMe	H	OMe	OMe
19 e	OMe	OMe	OBn	H
19 f	O C H_2 O		OMe	H

Figure 2.

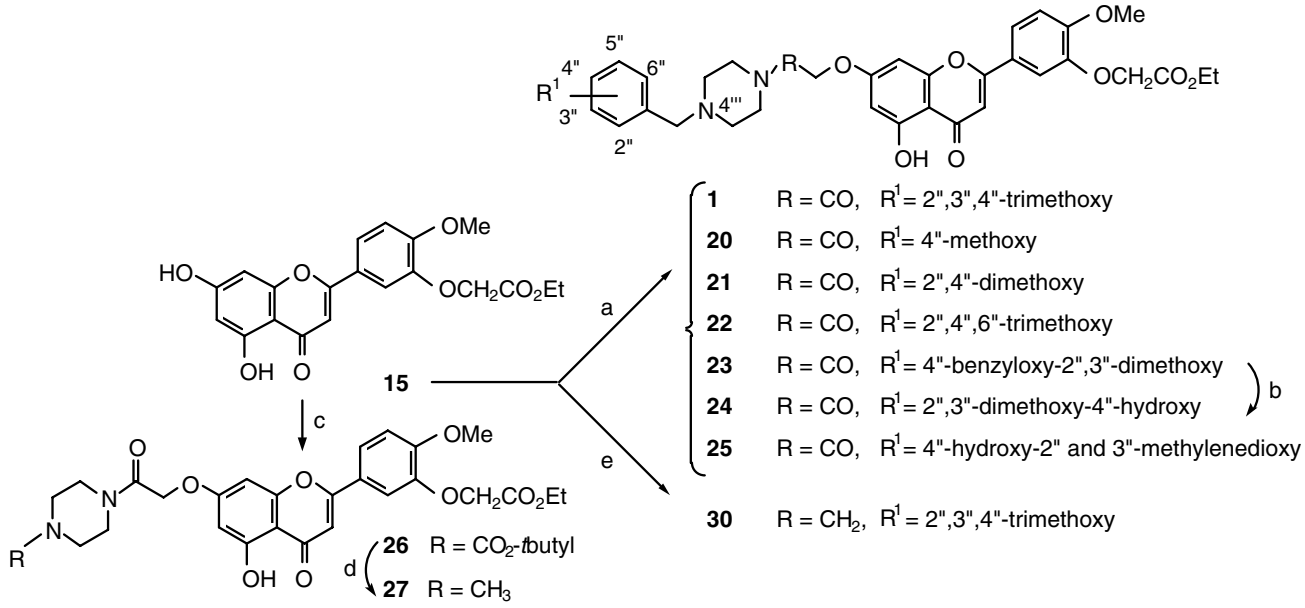
with the flavone **15** provided the compounds **20**–**23**, **25**, and **26**. **23** was subsequently transformed to **24** by catalytic hydrogenolysis, while **26** led to **27** by hydrolysis of the Boc function and then N-methylation; finally, the quaternized derivatives **28** and **29** were prepared from **1** by reaction of the 4‴-tertiary amine group, respectively, with *meta*-chloroperbenzoic acid and methyl iodide. As easy access to **30** by selective reduction of **1** seemed uncertain, then we chose to synthesize **30** by the following two-step procedure: reductive amination of 2,3,4-trimethoxybenzaldehyde by *N*-(2-hydroxyethyl)piperazine ($NaBH_3CN$ in AcOH) giving the crude benzylpiperazine which was reacted with **15** under Mitsunobu conditions (DEAD, PPh_3 in THF).[11]

Synthesis of analogs of **1** *by modification of the flavone moiety*. In the second part of the study, the chloroacetamide **19 a** (corresponding to the piperazinyl chain of **1**) was condensed with various flavonic moieties. Compounds **31**–**33** differed from **1** only in the ether group at C-3′, and they were obtained as **1** by replacement of flavone **15** by its analogs **16**–**18**[12] that were synthesized from diosmetin (**11**) in three steps (7-O-benzylation; 3′-O-alkylation; hydrogenolysis of the benzyl ether). Compounds **34**–**36** arose from the coupling of **19a** with easily available or previously prepared 7-hydroxyflavones.[13]

Antimalarial evaluation. The in vitro antiplasmodial activity of the new compounds was first assayed against the chloroquine-resistant FcB1 strain of *P. falciparum*,[5] and their toxicity was evaluated against the human diploid embryonic lung cell line MRC5.[8] Index of selectivity ($IS = IC_{50MRC5}/IC_{50\,P.\ falciparum}$) indicates the specificity of compounds against the parasite. The antiplasmodial evaluation of compounds **20**–**30** (Table 2), analogs of **1** modified on the piperazinyl chain, confirms the positive effect of a polymethoxylation pattern (**21**–**24** vs **20**, **25**), and the importance of a lipophilic character (quaternization of the piperazinyl chain in **28** and **29** greatly decreases the antiplasmodial activity). Unexpectedly, the antiplasmodial activity does not seem to be correlated with the basicity of compounds: carbamate **26** is much more potent than the *N*-methylpiperazinyl compound **27**, while reduction of the amide function weakens the activity (**30** vs **1**). The only compound within this series with a better activity than **1** is the phenolic analog **24**, but with a less favorable index of selectivity (IS > 140 and 10, respectively). All the other polyoxygenated patterns of the benzylpiperazinyl chain (compounds **21**–**25**) resulted in much lower specificities toward the parasite, which accounts for the choice of the 2,3,4-trimethoxybenzylpiperazinyl moiety in the structure of the following synthesized compounds. The examination of the second series of analogs (Table 3) indicates that replacement of the ethoxycarbonylmethyl ether of **1** by some other ether groups at 3′ slightly decreases antiplasmodial activity (**31**–**33**). In other respects, an unsubstituted B ring on the flavone moiety seems to be detrimental (**34**, **35** vs **1**), while the lipophilic 3,5-di-*tert*-butyl-4-hydroxyphenol substitution pattern in **36** maintains the antiplasmodial activity with a good specificity (IC_{50} on *P. falciparum* 1.2 μM, IS > 125).

Considering these results, the four most active compounds (**1**, **24**, **31**, and **36**) have been evaluated on the chloroquine-sensitive strain Thai and the chloroquine-resistant strain K1 of *P. falciparum* (Table 4). The antiplasmodial potencies of compounds **24**, **31**, and **36** were homogeneous with a maximum difference of IC_{50} values of a factor 3 between the different strains. In contrast, compound **1** showed a marked weaker activity against the Thai and K1 strains compared to the FCB1 strain (IC_{50} 13 μM vs 1 μM, respectively). No apparent correlation was observed with the chloroquine resistance of strains tested. The antimalarial activity of compounds **1** and **36** was evaluated in vivo against mice infected by *Plasmodium yoelii nigeriensis*[14] (Table 5). A similar dose-dependent reduction of parasitemia was observed for both compounds at day 4 (around 40% of parasite growth inhibition at 50 mg/kg/day). However, no

Table 2. Synthesis, structure and in vitro antiplasmodial activities of compounds **20**–**30**



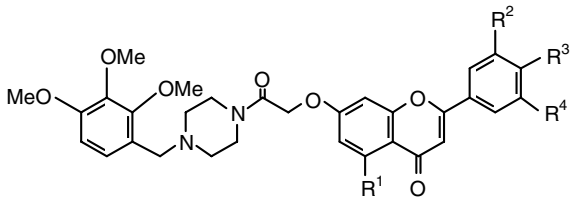
Compound	Formula	*P. falciparum* (FcB1 strain) IC_{50} (μM)	Cytotoxicity MRC5 IC_{50} (μM)	IS[a]
1	$C_{36}H_{40}N_2O_{12}$	1	>140	>140
20	$C_{34}H_{36}N_2O_{10}$	8.9	nd[b]	
21	$C_{35}H_{38}N_2O_{11}$	1.7	5.6	3.3
22	$C_{36}H_{40}N_2O_{12}$	1.9	9.5	5
23	$C_{42}H_{44}N_2O_{12}$	2.8	10	3.6
24	$C_{35}H_{38}N_2O_{12}$	0.6	10	16.7
25	$C_{35}H_{36}N_2O_{12}$	10	37	3.7
26	$C_{31}H_{36}N_2O_{11}$	1.8	3.8	2.1
27	$C_{27}H_{30}N_2O_9$	26	nd	
28(**1**, *N*-oxide)	$C_{36}H_{40}N_2O_{13}$	19	nd	
29(**1**, iodomethylate)	$C_{37}H_{43}$ I N_2O_{12}	41	nd	
30	$C_{36}H_{42}N_2O_{11}$	4.3	18	4.2

Reagents and conditions: (a) $KHCO_3$, **19a**–**f** in DMF (120 °C, 2.5 h); (b) H_2, Pd–C in MeOH (rt, 1 atm, 3 h); (c) $KHCO_3$, 1-Boc-4-chloroacetyl-piperazine in DMF (120 °C, 2.5 h); (d) CH_2Cl_2–TFA 1–1 (rt, 1 h); CH_2O, $NaBH_3CN$ in AcOH (rt, 2 h); (e) 1-(2-hydroxyethyl)-4-(2,3,4-trimethoxybenzyl)piperazine, $P(Ph)_3$, DEAD in THF (rt, 15 h). Chloroquine was used as positive control (IC_{50} 0.2 μM).

[a] Index of selectivity defined by the ratio IC_{50} on MRC5 cells/IC_{50} on *P. falciparum*.

[b] Not determined. Determinations were means of three independent experiments.

Table 3. Structure and in vitro antiplasmodial activity of compounds **31**–**36**



Compound	R^1	R^2	R^3	R^4	Formula	*P. falciparum* (FcB1 strains) IC_{50} (μM)	Cytotoxicity (MRC5 cells) IC_{50} (μM)	IS[a]
31	OH	H	OMe	OCH_2CO_2*tert*-Butyl	$C_{38}H_{44}N_2O_{12}$	1.7	>140	>82
32	OH	H	OMe	*O*-Hexyl	$C_{38}H_{46}N_2O_{10}$	3.9	nd[b]	
33	OH	H	OMe	*O*-Cyclopentyl	$C_{37}H_{42}N_2O_{10}$	2.8	>150	>53
34	H	H	H	H	$C_{31}H_{32}N_2O_7$	13	nd	
35	OH	H	H	H	$C_{31}H_{32}N_2O_8$	14	nd	
36	H	*tert*-Butyl	OH	*tert*-Butyl	$C_{39}H_{48}N_2O_8$	1.2	>150	>125

Chloroquine was used as positive control ((IC_{50} 0.2 μM).

[a] Index of selectivity defined by the ratio IC_{50} on MRC5 cells/IC_{50} on *P. falciparum*.

[b] Not determined. Determinations were means of three independent experiments.

Table 4. In vitro antiplasmodial activities of products on chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*

Compound	FcB1 strain IC_{50} (μM)	Thai strain IC_{50} (μM)	K1 strain IC_{50} (μM)
1	1	13	13
24	0.6	1.2	0.4
31	1.7	1.5	1.4
36	1.2	0.9	0.4
CQ	0.2	0.014	0.27

Chloroquine (CQ) was used as positive control. Determinations were means of three independent experiments.

Table 5. In vivo antimalarial activities of compounds **1** and **36**[a]

Doses[b] (mg/kg)	**1**		**36**	
	% inhibition[c]	DEAD[d]	% inhibition[c]	DEAD[d]
10	27.1 ± 9.0	2/5	24.1 ± 5.3	5/5
50	43.1 ± 14.1	2/5	38.5 ± 12.1	4/5

[a] Assays were performed on *Plasmodium yoelii nigeriensis* N67 infected mice according to the 4-day suppressive test described by Peters et al.[14]

[b] Drugs were administered orally at 0.5 ml/mouse/day

[c] % inhibition was determined by comparison of the parasitemia measured in treated mice to that of non-treated mice at day 4.

[d] Number of dead mice at day 14 (5 mice per group). All mice were dead in the non-treated group at day 14. Chloroquine (10 mg/kg/ml) was used as control and cured totally the mice from parasites.

difference of mice survival was observed between the non-treated group and the group treated with compound **36**. In contrast, a significant increase of mice survival was constantly observed with compound **1**.

In conclusion, this pharmacomodulation study showed that the marked in vitro antiplasmodial potency of some of the compounds comes from the combination of two moieties, which are individually of weak activity. A same observation had already been noticed in this series for the MDR modulation.[1] One of the synthesized compounds exhibits, though in a medium range, an antimalarial effect in vivo. Until now, only few flavonoids, such as licochalcone A,[15,16] were reported to have in vivo antimalarial activity. Interestingly, most of the studied compounds were semi-synthesized from diosmin, an easily available *Citrus* flavonoid, which confirms the interest of natural products as raw materials for medicinal chemistry.

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