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Synthesis of novel α-pyranochalcones and pyrazoline derivatives as *Plasmodium falciparum* growth inhibitors

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ABSTRACT

Both the lack of a credible malaria vaccine and the emergence and spread of parasites resistant to most of the clinically used antimalarial drugs and drug combination have aroused an imperative need to develop new drugs against malaria. In present work, α-pyranochalcones and pyrazoline analogs were synthesized to discover chemically diverse antimalarial leads. Compounds were tested for antimalarial activity by evaluation of the growth of malaria parasite in culture using the microtiter plate based SYBR-Green-I assay. The (*E*)-3-(3-(2,3,4-trimethoxyphenyl)-acryloyl)-2H-chromen-2-one (**Ga6**) turned out to be the most potent analog of the series, showing IC_{50} of 3.1 μg/ml against chloroquine-sensitive (**3D7**) strain and IC_{50} of 1.1 μg/ml against chloroquine-resistant field isolate (**RKL9**) of *Plasmodium falciparum*. Cytotoxicity study of the most potent compounds was also performed against **HeLa** cell line using the MTT assay. All the tested compounds showed high therapeutic indices suggesting that they were selective in their action against the malaria parasite. Furthermore, docking of **Ga6** into active site of falcipain enzyme revealed its predicted interactions with active site residues. This is the first instance wherein chromeno-pyrazolines have been found to be active antimalarial agents. Further exploration and optimization of this new lead could provide novel, antimalarial molecules which can ward off issues of cross-resistance to drugs like chloroquine.

Malaria, a major cause of morbidity and mortality affecting third-world countries,[1] is one of the foremost health and developmental challenges facing mankind. Malaria is responsible for up to 500 million clinical cases and 3 million deaths annually.[2] The WHO estimates that malaria kills an African child every 30 s.[3] In the absence of an effective malaria vaccine, prevention and treatment relies mainly on drugs. Even as a number of drugs and drug combinations are in clinical use for malaria, many of these are becoming less effective due to widespread parasite resistance (like recent cases of falciparum malaria resistance to artesunate–mefloquine combination).[4] This means that there is an imperative need to develop new drugs with different mechanisms of action to help preclude issues of cross-resistance.

Chalcone is an exceptional chemical template having multifarious biological activities. Antimalarial property of some chalcone derivatives is derived from their ability to inhibit the parasitic cysteine proteases like the falcipain.[5] These proteases catabolize globin into small peptides within the acidic food vacuole of the intra-erythrocytic malaria parasite. Without cysteine protease action osmotic swelling occurs, food vacuolar functions are impaired, and the parasite is starved to death.

The literature on chalcones (Fig. 1) as antimalarials was analyzed to provide a meaningful overview of the structural requirements for chalcones as antimalarials. Some of the important points to be considered are[5,6] (i) the influence of B ring on the activity is related to size considerations and bulky substitutions are favorable, while the A ring may be more important in influencing hydrophobicity; (ii) presence of chloro and fluoro substitutions on ring A does not necessarily increase the activity and the influence of these halogen atoms on ring A seems to depend on the kind of substitution on ring B; (iii) Steric and hydrophobic factors, particularly substituent on ring A must be width–limited so that the molecular width along *X*-axis of chalcone derivatives is small; (iv) high electron density at the C_3, C_4 of ring B might play a favorable role in activity; (v) requirement of hydrogen bond acceptor at C_4 of ring B in a particular orientation to provide stronger and effective hydrogen bonding.

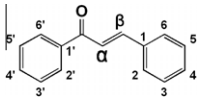

Figure 1. General structure of chalcone.

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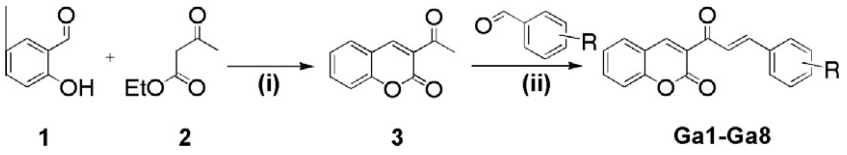
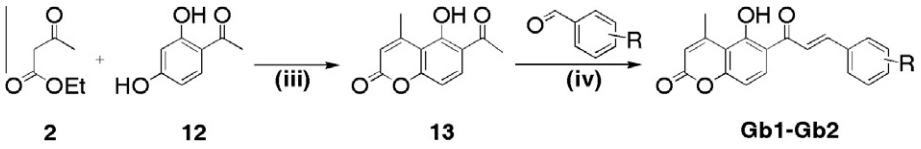
As a result, it was decided to undertake the synthesis and antimalarial evaluation of α-pyranochalcones. The acetophenone part in chalcone was replaced with the acetyl coumarines in α-pyranochalcones. This was done in order to satisfy the structural requirements of limited bulk and availability of hydrogen bond acceptor at C_4 of ring B.

The α-pyranochalcones **Ga1**–**Ga8** were synthesized from 3-acetyl-coumarin **3** (Scheme 1). The latter was prepared from salicylaldehyde **1** using a reported method,[7] with necessary modification to improve yield and appearance. This was followed by condensation of 3-acetyl-coumarin **3** with substituted benzaldehydes by using novel solvent-free method involving a heterogeneous catalyst, silica sulfuric acid (synthetic details are provided in Supplementary material). The α-pyranochalcones **Gb1**–**Gb2** were synthesized from 6-acetyl-5-hydroxy-4-methyl-2H-chromen-2-one **13**. Compound **13** was synthesized using Pechmann condensation as reported by Sethna et al.[8] (Scheme 2). Treatment of α-pyranochalcone **Ga3** with hydrazine hydrate and phenyl hydrazine in presence of acetic acid[9] afforded pyrazolines, **Gc** and **Gd** (Scheme 3). Similarly, pyrazoline **Ge** was synthesized from diphenylchalcone **20**. The *p*-hydroxyacetophenone in base catalyzed Claisen–Schmidt condensation with *p*-flourobenzaldehyde afforded the diphenylchalcone **20** (Scheme 4).[10] The synthesized compounds are summarized in Table 1.

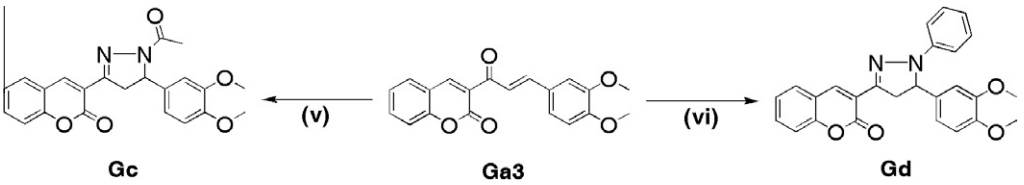
The antimalarial activity of all of the compounds was determined by SYBR-Green-I in vitro assay.[11,12] The results are shown in Table 2 indicate that eight of the 13 compounds evaluated, showed significant antimalarial activity against the chloroquine-sensitive (**3D7**) strain. Interestingly, six of these eight compounds showed good promise (IC_{50} < 15 μg/ml) also against **RKL9**, the chloroquine-resistant field isolate of *P. falciparum*. It is noteworthy that the resistance index of all the tested compounds is higher than 1 (for **Ga6**, its 2.8). This is due to their surprisingly higher potencies against chloroquine-resistant strain than that against chloroquine-sensitive one (Table 2). The same is not true for most of the previously reported chalcones and even for the artemisinin derivatives. The compound **Ga1** (IC_{50}^{3D7} = 3.1 μg/ml; IC_{50}^{RKL9} = 1.9 μg/ml) and **Ga6** (IC_{50}^{3D7} = 3.1 μg/ml; IC_{50}^{RKL9} = 1.1 μg/ml) were the most potent analogs of the series. The most active compounds (IC_{50}^{RKL9} < 10 μg/ml, i.e., **Ga1**, **Ga2**, **Ga6**, **Gc**, and **Gd**) were further evaluated for cytotoxicity by MTT assay method[13] against **HeLa** cell line. All tested compounds were found to be devoid of cytotoxicity at inhibitory concentrations. In general, their cytotoxicity appeared to be at much higher concentrations ($TC_{50}^{HeLa} \geqslant 100$ μg/ml) than the concentrations responsible for their antimalarial activity. This indicates their safety in the mammalian system. Furthermore, these compounds were found to possess good therapeutic indices ($TC_{50}^{HeLa}/IC_{50}^{RKL9}$ = 10–90.9) showing their selectivity towards malaria parasite.



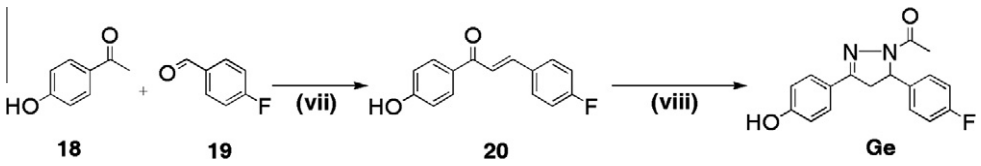
Scheme 1. Synthesis of 3-acetyl coumarine (**3**) and its chalcones (**Ga1**–**Ga8**). Reagents and condition: (i) piperidine, ethanol, rt, stirring; (ii) silica sulfuric acid, solvent-free.



Scheme 2. Synthesis of 6-acetyl-5-hydroxy-4-methyl-coumarin (**13**) and its chalcones (**Gb1**–**Gb2**). Reagents and conditions: (iii) $AlCl_3$/dry $PhNO_2$, 125–130 °C; (iv) silica sulfuric acid, solvent-free, 80 °C.



Scheme 3. Synthesis of α-pyranopyrazoline (**Gc**–**Gd**). Reagents and conditions: (v) hydrazine hydrate, glacial acetic acid, reflux; (vi) methanol, phenyl hydrazine, few drops of acetic acid, reflux.



Scheme 4. Synthesis of diphenyl chalcone (**20**) and its pyrazoline (**Ge**). Reagents and conditions: (vii) NaOH/dry methanol, rt, stirring; (viii) hydrazine hydrate, glacial acetic acid, reflux.

Table 1
Molecular formula, molecular weight, melting point and% yields of synthesized compounds

Compound code	R	Molecular formula	Molecular weight	Melting point (°C)	Yield (%)
Ga1	3,4,5-TriCH_3O–	$C_{21}H_{18}O_6$	366.36	155–156	75.88
Ga2	3-MeO-4-H–	$C_{19}H_{14}O_5$	322.31	171–172	79.12
Ga3	3,4-DiMeO–	$C_{20}H_{16}O_5$	336.34	151–152	74.4
Ga4	4-MeO–	$C_{19}H_{14}O_4$	306.31	153–154	79.98
Ga5	2,5-DiMeO–	$C_{20}H_{16}O_5$	336.34	114–115	72.0
Ga6	2,3,4-TriMeO–	$C_{21}H_{18}O_6$	336.34	131–132	74.64
Ga7	2,4,5-TriMeO–	$C_{21}H_{18}O_6$	366.36	155–156	67.69
Ga8	2-MeO–	$C_{19}H_{14}O_4$	306.31	143–144	68.55
Gb1	2-Cl	$C_{19}H_{13}ClO_4$	340.76	195–198	61.05
Gb2	2,5-DiMeo	$C_{21}H_{18}O_6$	366.36	160–162	69.6
Gc	[a]	$C_{22}H_{20}N_2O_5$	392.4	210–212	81.8
Gd	[a]	$C_{26}H_{22}N_2O_4$	426.46	148–150	77.42
Ge	[a]	$C_{17}H_{13}FN_2O_2$	296.3	228–231	71.88

[a] Structures are provided in Schemes 3 and 4.

Table 2
The in vitro antimalarial activity against chloroquine-sensitive (**3D7**) strain and chloroquine-resistant field isolate (**RKL9**) of *P. falciparum*; and cytotoxicity against **HeLa** cell line

Compound	Antimalarial activity against *P. falciparum* (μg/ml)				Cytotoxic activity against **HeLa** cell line (μg/ml)	
	IC_{50}^{3D7}	IC_{80}^{3D7}	IC_{50}^{RKL9}	Resistance index ($IC_{50}^{3D7}/IC_{50}^{RKL9}$)	TC_{50}^{HeLa}	$TC_{50}^{HeLa}/IC_{50}^{RKL9}$
Ga1	3.1	6	1.9	1.6	>100	>50
Ga2	11	24	4.9	2.2	>100	>20
Ga3	26.7	100	a	—	a	—
Ga4	>100	a	a	—	a	—
Ga5	2.7	11.3	a	—	a	—
Ga6	3.1	6.2	1.1	2.8	100	90.9
Ga7	>100	a	a	—	a	—
Ga8	>100	a	a	—	a	—
Gb1	20	25	13.5	1.5	a	—
Gb2	>100	a	a	—	a	—
Gc	10	14	9	1.1	>100	>10
Gd	10	44	7.6	1.3	>100	>13
Ge	>100	a	a	—	a	—
CQ[b]	20.63[c]	36.11[c]	206.37[c]	0.1	a	—
Artemisinin	4.51[c]	8.46[c]	4.51[c]	1	a	—

[a] Not done.
[b] Chloroquine diphosphate.
[c] ng/ml.

The antimalarial activity of chalcones with different substitution patterns has been previously reported.[14–18] Albeit, they were found to act through a variety of different mechanisms,[14–18] the inhibition of falcipain by newly designed chromenochalcones was apparent from the docking study of most active compound **Ga6** in the active site of falcipain enzyme. The docking of most active compound, **Ga6** was performed by GLIDE[19] module of Schrödinger Suite 2009 with protein, falcipain enzyme (PDB ID: 3BPF). The default settings with standard precision mode in the GLIDE module were used for docking. It can be inferred from the docking study that the compound **Ga6** seems to possess good affinity for active site residues of 3BPF. The Fig. 2 depicts the type of predicted interaction and fit of the molecule with the surface cleft of falcipain enzyme. The hydrogen bonding interaction with Cys 42 as predicted by the docked pose of **Ga6** is in agreement with the previously discussed requirement of hydrogen bonding acceptor in ring B.

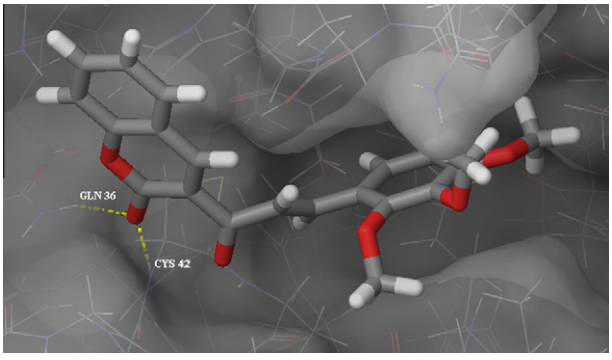


Figure 2. The proposed interactions of **Ga6** with active site residue Cys 42 of falcipain enzyme revealed from docking study.

The high activity of trimethoxy-derivatives (**Ga1** and **Ga6**) is also in accord with discussed influence of hydrophobicity and steric bulk on the ring A. Among the disubstituted compounds, the monohydroxylated compound **Ga2** is more active than the dimethoxy-derivatives. Between the 6-acetyl-coumarin derivatives, the halogenated derivatives (**Gb1**, IC_{50}^{RKL9} = 13.5 μg/ml) exhibited greater activity than the methoxylated derivative (**Gb2**, IC_{50}^{3D7} > 100 μg/ml). These results offer new possibilities for further improvements in the antimalarial performance of α-pyranochalcones derivatives. Although this initial study involved only a limited number of compounds, it has provided a platform that is well worth studying for further detailed SAR of pyranochalcones.

This is the first report wherein chromeno-pyrazolines were found to be potent antimalarials. Among the pyrazolines, diphenyl pyrazoline **Ge** possessed insignificant antiplasmodial potential, whereas α-pyranopyrazolines, **Gc** and **Gd** were found to be significantly active against both strains of the malarial parasite. This

suggests that as new scaffolds, α-pyranopyrazolines can further be explored and optimized as novel lead molecules for their antimalarial activity.

These results revealed that the compounds synthesized by us exhibited promising antimalarial activity against both chloroquine-sensitive strain (**3D7**) and chloroquine-resistant field isolate (**RKL9**) of *P. falciparum* with meaningful SAR. A comparatively higher potency against **RKL9** than that against **3D7** strain of the two novel α-pyranopyrazolines in the present study suggests that active α-pyranopyrazoline leads can be further optimized for building potent and chemically diversified antimalarial drugs with less chances of developing cross-resistance.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.069.

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