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Conformationally restricted anti-plasmodial chalcones

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Abstract—Chalcones can exist as *Z*- or *E*-isomers and it is generally anticipated that both isomers are equipotent. In order to determine the active isomer of anti-plasmodial chalcones a series of analogues locked in the *Z*- or the *E*-form were prepared and evaluated for their anti-plasmodial activity. It was shown that the *Z*-locked analogue was nearly inactive, whereas the *E*-locked analogues were equipotent to the parent chalcones, indicating that the *E*-isomer is the active conformation.

A large number of publications covering bioactive chalcones appear every year.[1] The compounds have shown an array of pharmacological activities, such as anti-protozoal,[2–4] anti-inflammatory,[5] immunomodulatory,[6] nitric oxide inhibition,[7] inhibition of the production of interleukin-1[8] and anti-cancer activities.[9] Licochalcone A (LicA; Table 1) has been used extensively as a model compound in anti-parasitic research.[2,10,16,25,39]

Chalcones exist as either the *E*- or the *Z*-isomers (Chart 1). The *E*-isomer is in most cases the thermodynamically most stable form and consequently, the majority of the chalcones is isolated as the *E*-isomer; in general, recrystallisation of an *E*–*Z* mixture yields the *E*-isomer as the only stereoisomer. It has previously been shown that the α,β-double bond is very labile for photoisomerisation in solution, giving a mixture of the *E*- and *Z*-isomers.[11,26–28] Indeed, we have observed this in our in vitro studies (e.g., in an assay for microsomal turn-over) as well as our vivo studies.

A few studies have addressed this problem. It has been shown that the rate of the isomerisation and the equilibrium ratio depend on the substitution in the aromatic rings and the solvent used.[12–14] The data indicate that electron donating groups and the chelating properties of the substituents in the 2′-position have a significant influence on the degree of isomerisation.[13,14] However, these rules only apply for some very specific compounds, substituents and positions. From our experience, having synthesised more than 1000 different chalcones, it is almost impossible to predict which chalcones are prone to photoisomerisation and to what extent.

It has previously been anticipated that the two stereoisomers are equipotent but no proof has been published. If the *E*- and the *Z*-isomers are equipotent, the interpretation of the biological data should be straightforward, since the degree of isomerisation would be without effect on the activity of the compounds. On the contrary, if there is a marked difference between the activity of the *E*- and *Z*-forms, the observed activity is a combination of the intrinsic activity as well as the photolability of the active isomer. As isomerisation can be extremely fast (seconds), the biological evaluation of the compounds can be quite complicated.

To the best of our knowledge, only the studies by Iwata et al., [14] and Shibata[15] have attempted to investigate the biological activity of *E*- and *Z*-chalcones, respectively. The two isomers were isolated by HPLC and assayed for their biological activity. The authors observe a relatively small difference in the activity of the isolated isomers leading to the conclusion that the isomers are equipotent. On the basis of the work presented in this communication, we believe that the isolated isomers must have converted back to an equilibrium mixture during the time of the assay.

This study seeks to determine the anti-plasmodial activities of the *E*- and *Z*-isomers in order to identify the active isomer. This is achieved by the synthesis and

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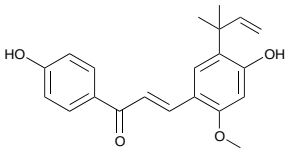
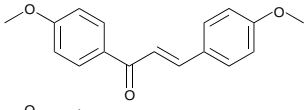
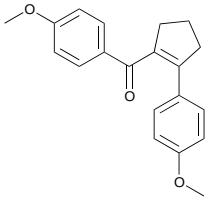
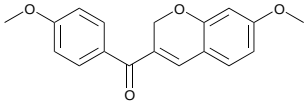
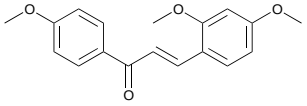
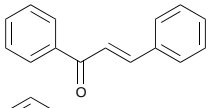
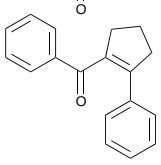
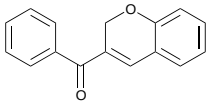
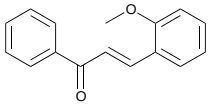
Table 1. In vitro anti-malarial activity[29] of the prepared analogues against *P. falciparum* 3D7[a]

Compound	Structure	IC_{50} (μg/ml) *P. falciparum*
LicA		2.2
1[2,17]		8.9
5[18]		>100
11[33]		13.3
16[31]		6.0
2[2]		6.7
6[30]		35
12[22]		4.7
17[32]		7.6

[a] Each value represents the mean of three experiments.

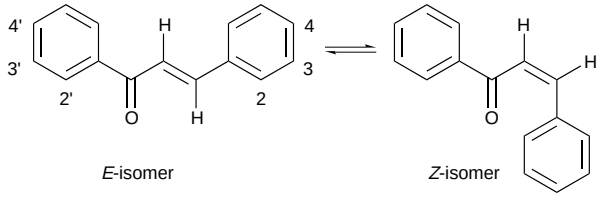
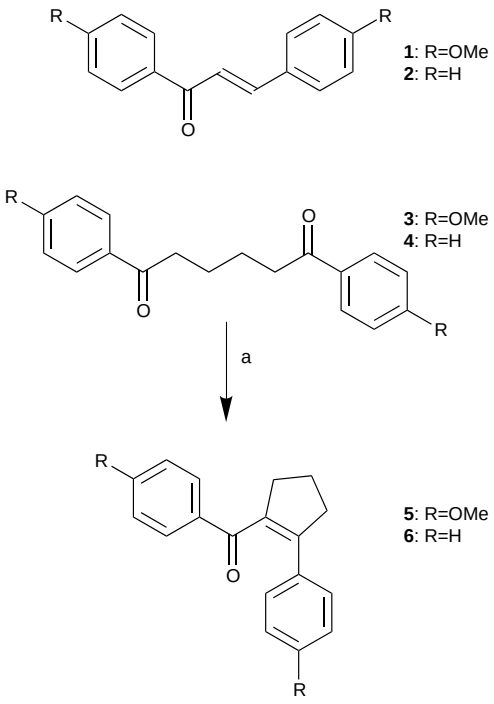


Chart 1.

testing of conformationally restricted *Z*- and *E*-analogues of different known anti-plasmodial chalcones.

The chalcones (**1, 2**, **18–20**) were prepared by classical Claisen–Schmidt condensation using a catalytic amount of sodium hydroxide in ethanol.[19]



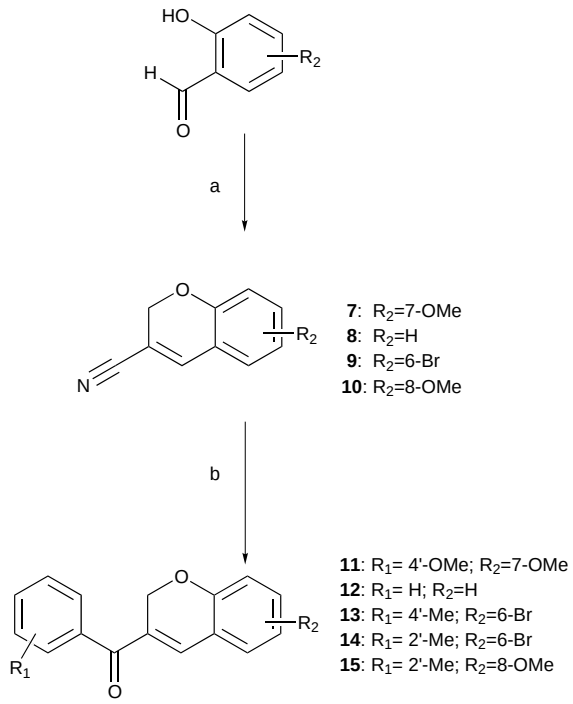
Scheme 1. Reagents and conditions: (a) CH_3COOH, concd HCl, 50 °C.

The *Z*-locked analogues **5** and **6** of the chalcones **1** and **2** were prepared from the diketones **3** and **4**, respectively, by acid-catalysed cyclization using concentrated hydrochloric acid in acetic acid (Scheme 1). The *E*-locked analogues were prepared in two steps. Treatment of an appropriate substituted 2-hydroxy-benzaldehyde with acrylonitrile in the presence of catalytic amount of DABCO gave **7–10**.[20,21] Subsequent treatment with aryl magnesium bromide gave the *E*-locked chalcone analogues **11–15** (Scheme 2).[22]

Table 1 summarises the anti-plasmodial activity of two series of *E*- and *Z*-conformationally restricted analogues and their parent chalcones against *Plasmodium falciparum*.

Parent chalcones **1** and **2** show good activity against the *Plasmodium* parasites. On the contrary, the conformationally restricted analogues **5** and **6** are nearly inactive. Unfavourable substitution of the α,β-double bond or conformational factors can explain the loss of activity of the compound. Previously it was shown that α- or β-substitution with alkyl groups only marginally influences the anti-parasitic activity of the chalcones.[23] These results strongly indicate that the loss of activity is due to the *Z*-conformation of the molecule.

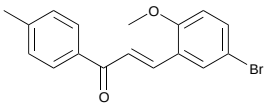
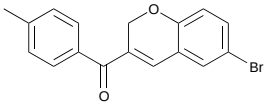
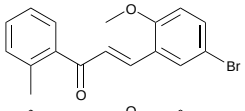
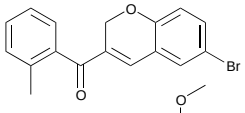
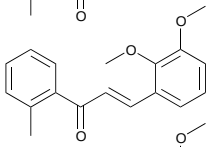
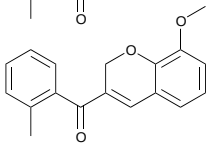
Similar to the *Z*-locked analogues **5** and **6**, we have prepared the corresponding conformationally restricted *E*-locked analogues (**11**, **12**). The data in Table 1 show that the *E*-locked analogues are as potent as the parent chalcones against *Plasmodium* parasites, thereby indicating that the bioactive isomer of anti-parasitic chalcones is the *E*-isomer.



Scheme 2. Reagents and conditions: (a) acrylonitrile (4.5 equiv), DABCO (0.1 equiv), neat, 80 °C, 18 h; (b) i: Ar-MgBr (1.2 equiv) diethyl ether, reflux, 6 h. ii: 2 M HCl.

To further support this observation, three more *E*-conformationally locked analogues of known anti-plasmodial chalcones were prepared (Table 2). The data show that the *E*-locked analogues were equipotent to the parent chalcones, supporting the above conclusion. In addition, a novel and promising class of anti-plasmodial compounds has been discovered.

Table 2. In vitro anti-malarial activity[29] of the prepared analogues against *P. falciparum* 3D7[a]

Compound	Structure	IC_{50} (μg/ml) *P. falciparum*
18[24]		10.0
13[34]		8.6
19[37]		9.3
14[35]		13.5
20[38]		11.0
15[36]		15.1

[a] Each value represents the mean of three experiments.

We believe that the data shown in this report are an important finding in regard to the biological properties of chalcones. Clearly, our findings provide insight into the potential difficulties of working with chalcones that can easily undergo isomerisation on exposure to light. We recommend that scientists working with chalcones take the presented results into account and make sure that the compounds are not exposed to light and to keep solutions in dark glass vials.

We have prepared conformationally restricted analogues of anti-plasmodial chalcones. The analogues with the double bond in the *Z*-conformation were nearly inactive, whereas the corresponding analogues being locked in the *E*-conformation were equipotent to the parent chalcones against *Plasmodium* parasites. This supports the hypothesis that the *E*-isomer of the chalcones is the active one with regard to anti-plasmodial activity.

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30. (a) Hoffman, W. D.; McEwen, W. E.; Kleinberg, J. *Tetrahedron* **1959**, *5*, 293; (b) For **6**: ^{1}H NMR ($CDCl_3$): δ = 7.74 (d, J = 6.9 Hz, 2H), 7.41–7.32 (m, 1H), 30–7.20 (m, 2H), 7.18–7.05 (m, 5H), 3.08–2.86 (m, 4H), 2.20–2.05 unitet, 2H.
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33. For **11**: ^{1}H NMR ($CDCl_3$): δ 7.66 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 8.8 Hz, 2H), 6.71 (d, J = 1.3 Hz, 1H), 6.43 (dd, J = 8.4 Hz, J = 2.5 Hz, 1H), 6.39 (d, J = 1.8 Hz, 1H), 5.06 (d, J = 1.4 Hz, 2H), 3.82 (s, 3H), 3.74 (s, 3H).
34. For **13**: ^{1}H NMR($CDCl_3$): δ 7.64 (2H, d, J = 8.3 Hz), 7.34 (1H, dd, J = 8.1 Hz, 2.4 Hz), 7.30 (2H, d, J = 8.3 Hz), 7.22 (1H, d, J = 2.4 Hz), 7.03 (1H, br s), 6.79 (1H, d, J = 8.1 Hz) 5.18 (2H, br s), 2.42 (3H, s).
35. For **14**: ^{1}H NMR($CDCl_3$): δ 7.39 (1H, td, J = 7.4 Hz, 1.8 Hz), 7.33 (1H, dd, J = 8.8 Hz, 2.6 Hz), 7.30–7.23 (3H, m), 7.17 (1H, d, J = 2.6 Hz), 6.82 (1H, br s), 6.77 (1H, dd, J = 8.6 Hz, 0.6 Hz), 5.19 (2H, s), 2.36 (3H, s).
36. For **15**: ^{1}H NMR($CDCl_3$): δ 7.38 (1H, td, J = 7.5 Hz, 1.6 Hz), 7.31 (1H, dd, J = 7.5 Hz, 1.6 Hz), 7.30–7.22 (2H, m), 6.92–6.90 (2H, m), 6.86 (1H, t, J = 7.5 Hz), 6.68 (1H, dd, J = 7.5 Hz, 1.5 Hz), 5.15 (2H, s), 3.90 (3H, s), 2.38 (3H, s).
37. For **19**: ^{1}H NMR($CDCl_3$): δ 7.72 (1H, d, J = 16.3 Hz), 7.67 (1H, d, J = 2.6 Hz), 7.51 (1H, dd, J = 8.2 Hz, 1.6 Hz), 7.45 (1H, dd, J = 8.8 Hz, 2.6 Hz), 7.38 (1H, td, J = 7.3 Hz, 1.6 Hz), 7.3–7.24 (m, 2H), 7.20 (1H, d, J = 16.3 Hz), 6.82 (1H, d, J = 8.8 Hz) 3.82 (3H, s), 2.41 (3H, s).
38. For **20**: ^{1}H NMR ($CDCl_3$): δ 7.81 (1H, d, J = 16.1 Hz), 7.81 (1H, bd, $J \approx$ 8 Hz), 7.32 (1H, td, J = 7.9, 1.8 Hz), 7.30–7.20 (3H, m), 7.18 (1H, d, J = 16.1 Hz), 7.08 (1H, t, J = 7.9 Hz), 6.96 (1H, dd, J = 8.0, 1.3 Hz), 3.87 (3H, s), 3.82 (3H, s), 2.46 (3H, s).
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