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Synthesis and biological evaluation of chalcones as inhibitors of the voltage-gated potassium channel Kv1.3

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Abstract—Chalcone derivatives of the natural product khellinone were synthesised and screened for bioactivity against the voltage-gated potassium channel Kv1.3. X-ray crystallography was employed to investigate relationships between the structure and function of a selection of the reported chalcones.
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Chalcones are a group of naturally occurring compounds, found in various plant species, comprising two aryl rings linked by an α,β-unsaturated ketone. Chalcones have been reported with a wide variety of biological activities, including antiparasitic,[1,2] antibacterial,[3] antifungal,[4] anticancer[5,6] and antiinflammatory activity.[7] Due to this diversity of bioactivity, chalcone could be considered a 'privileged structure', as described by Evans and coworkers.[8] Whilst chalcones are active against various protein targets, modification of the privileged core could lead to novel compounds with specifically targeted inhibitory activity.

Enones with aromatic groups attached to the carbonyl, as in chalcones, have a preference for the s-*cis* conformation (Fig. 2),[11] where the carbonyl and olefin are on the same side of the single bond joining these two groups, in order to reduce the unfavourable interaction which would occur in the s-*trans* conformation, between a β-hydrogen and a hydrogen *ortho* to the carbonyl on the aromatic ring. Alkylation at the α-position increases steric bulk across the single bond, forcing the chalcone to adopt an s-*trans* conformation (Fig. 2). A number of research groups have attempted to exploit this property of chalcones to improve the potency of bioactive chalcones.

Antimitotic agents such as colchicine (**1**, Fig. 1) and combretastatin A4 (**2**, Fig. 1) have served as the template for the design of highly oxygenated chalcones with anti-cancer activity.[6,12–14] Ducki and coworkers have reported on the synthesis of antimitotic α-methyl chalcones like **3** (Fig. 1), describing these compounds as having up to 20-fold increase in potency over chalcones that are unsubstituted at the α-position.[12] They attribute this increase in potency to the s-*trans* geometry being favourable for activity over the s-*cis* conformation. Edwards and coworkers have also reported on α-methyl and α-bromo chalcones with a twofold increase in antimitotic activity over unsubstituted chalcones.[15] The observed improvement in bioactivity may be attributed to the enone geometry.

Amongst the chalcones with antiparasitic activity,[1,2,10,16,17] Nielson and coworkers have described antimalarial and antileishmanial chalcones that are analogues of Licochalcone A (**4**, Fig. 1).[10] They determined that α-alkylation had little effect on the antiprotozoal activity of these chalcones, and suggest that the enone moiety acts as a spacer between the two pharma-

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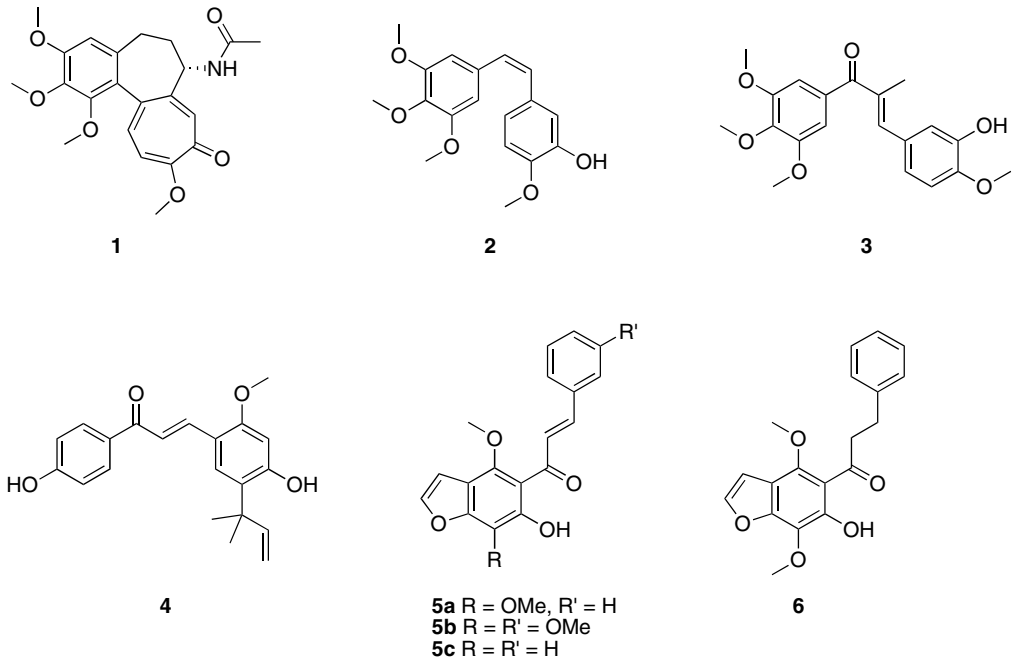
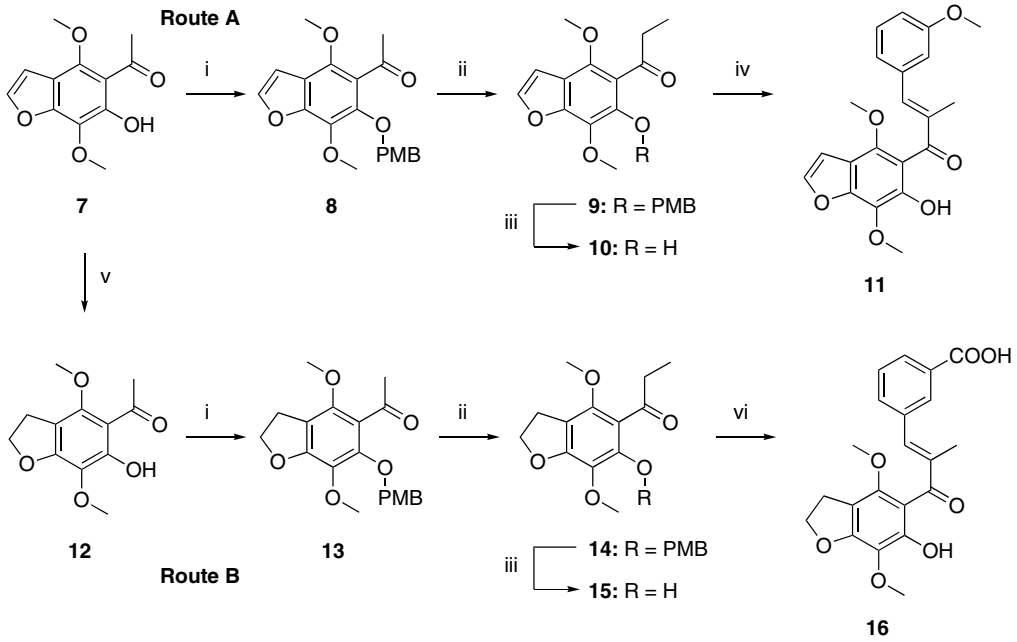


Figure 1. Highly oxygenated chalcones and related compounds with biological activity.[6,9,10]

cophore aromatic rings. A decrease in antimalarial activity in chalcones with α-substitution has been reported by Li and coworkers.[2] They describe the enone as a rigid spacer, and that conjugation is required for activity, as reduced potency is observed with reduction of the α,β-double bond.[2]

We have previously reported chalcones **5a** and **b** (Fig. 1), derivatives of the natural product khellinone (**7**, Scheme 1), which possess submicromolar blocking activity against the voltage-gated potassium channel Kv1.3.[9] This ion channel is implicated in the pathogenesis of autoimmune disorders such as multiple sclerosis, type I diabetes mellitus and psoriasis.[18–20] We have also described the reduced derivative **6**, which is equipotent to **5a** in blocking Kv1.3, thus suggesting that covalent modification of the enone is not relevant to the Kv1.3 activity of these compounds.[9]

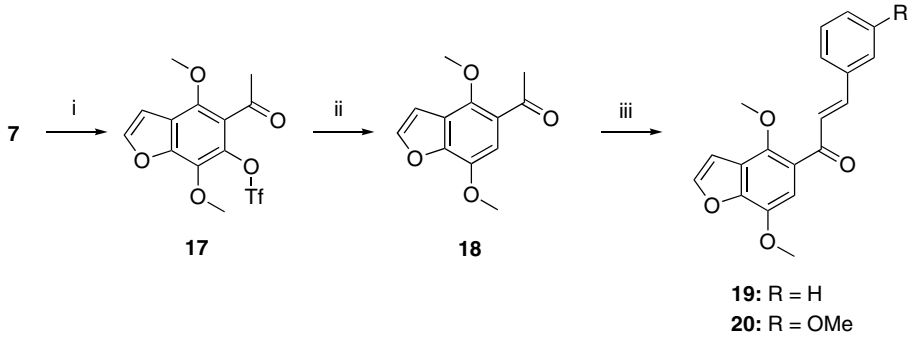


Scheme 1. Reagents and conditions: (i) *p*-methoxybenzyl chloride, Cs_2CO_3, DMF, 50 °C, (**8** 89%, **13** 98%); (ii) *n*-butyllithium, *i*-Pr_2NH, THF, DMPU, −78 °C, then MeI, −25 °C, (**9** 35%, **14** 70%); (iii) I_2, MeOH, 50 °C, (**10** 59%, **15** 84%); (iv) 3-methoxybenzaldehyde, 3 M NaOH, MeOH, 60 °C, 52%; (v) 10% Pd/C, H_2, MeOH, 86%; (vi) 3-carboxybenzaldehyde, 3 M NaOH, MeOH, 50 °C, 54%.

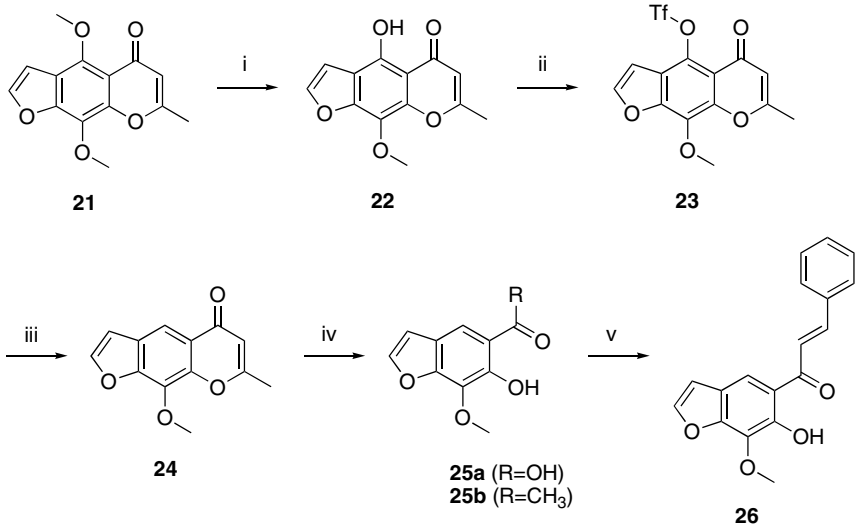
In the current work, we have chosen to investigate further the use of a chalcone group as a relatively rigid conformational SAR probe for the Kv1.3 blocking activity of khellinone derivatives. We prepare an α-methyl chalcone derivative of khellinone to determine whether the s-*trans* conformation is adopted and whether this favourably modulates channel-blocking activity, relative to the unsubstituted chalcone. In addition, we describe the activity of khellinone chalcones in which the oxygen substituents in the 4-, 6- or 7-position have been removed and hence may be metabolically more robust than their more highly oxygenated counterparts.[21,22]

The synthesis of α-methyl chalcones **11** and **16** is shown in Scheme 1. Compound **16** was prepared primarily for conformational analysis by X-ray crystallography. Inclusion of the carboxylic acid group gave a more crystalline compound than with the methoxy group. The furan was reduced for ease of synthesis, and it was assumed that this modification would have no effect on the enone geometry. The phenolic oxygen in khellinone (**7**) was protected as the PMB-ether to give **8**, which was treated with LDA and iodomethane to give the α-methylated ketone **9**. The poor yield in this step (35%) was due in part to the competitive methylation at the 2-position of the benzofuran. The PMB-group was deprotected with iodine in methanol and **10** was converted to the chalcone **11** by Claisen–Schmidt condensation. In order to prepare **16**, the furan of **7** was reduced via hydrogenation to give **12**. After PMB-protection of the phenol to give **13**, this compound was alkylated, affording **14** in 70% yield, double the yield compared with the benzofuran. After PMB-deprotection, **15** was converted to chalcone **16** by Claisen–Schmidt condensation.

We have previously reported the synthesis and bioactivity of **5c** (Fig. 1), a chalcone analogue of khellinone with the 7-methoxy group removed.[9] The 4-desmethoxy and 6-deshydroxy khellinone chalcones targeted in this work were prepared as follows. As outlined in Scheme 2, the phenol of **7** was activated as the triflate with triflic anhydride to give **17**. No reduction of the triflate was observed when $Pd(OAc)_2$, HCOOH, Et_3N and PPh_3 in DMF were used. On the basis of the accelerating effects of bidentate phosphine ligands,[23,24] triphenylphosphine was replaced with 1,3-bis(diphenylphosphino)propane (DPPP). This led to the reduction of the triflate **17** in excellent yield to give compound **18**, which was converted to the chalcones **19** and **20** via Claisen–Schmidt condensations with appropriate aldehydes.



Scheme 2. Reagents and conditions: (i) Triflic anhydride, 2,4,6-collidine, DMAP, DCM, −40 °C to rt, 97%; (ii) *n*-Bu_3N, DPPP, $PdCl_2(PPh_3)_2$, HCOOH, DMF, 80 °C, 89%; (iii) aldehyde, 3 M NaOH, MeOH, (**19** 48%, **20** 36%).



Scheme 3. Reagents and conditions: (i) BCl_3, DCM, −78 °C to rt, 91%; (ii) Tf_2O, Et_3N, DCM, 0 °C to rt, 70%; (iii) PPh_3, $Pd(OAc)_2$, Et_3N, HCOOH, DMF, 70 °C, 55%; (iv) 3 M NaOH, EtOH, 90 °C, **25a** 63%, **25b** 7%; (v) **25b**, 3-methoxybenzaldehyde, 1 M NaOH, MeOH, 0 °C to rt, 35%.

Table 1. EC_{50} values of khellinone chalcone analogues for block of Kv1.3

Compound	EC_{50} Jurkat[a] (μM)	EC_{50} L929[b] (μM)
5a	—	0.4
5b	0.9	0.8
5c	—	17
11	3.0	—
19	2.4	—
20	2.4	—
26	1.7	—

[a] Compound EC_{50} values were obtained by fitting a Hill equation to the normalised reduction of the Kv1.3 current integral at compound concentrations ranging from 10^{-9} to 10^{-5} M. All electrophysiology was performed using a planar patch clamp (Port-A-Patch, Nanion Technologies Gmbh, Munich) in contrast to conventional patch clamp.

[b] Assay conditions for patch clamp on L929, along with these results have been previously published.[9]

To prepare the 4-desmethoxy analogue, the methyl ether in the 4-position of khellin (**21**) was selectively cleaved using boron trichloride, affording **22** in excellent yield (Scheme 3). Activation to the triflate **23** and subsequent reduction to **24** were performed as described above, although the 4-position was reduced more readily than the 6-position triflate, and a bidentate ligand was not required. In contrast to the khellin system, which exclusively yields ketone **7** upon ring opening, hydrolysis of **24** gave mostly the carboxylic acid **25a** (63%) and only a small amount of the desired ketone **25b** (7%).[25] The chalcone **26** was prepared from **25b** as described above.

The EC_{50} values for inhibition of Kv1.3 currents were determined using the whole cell mode with human Jurkat cells which endogenously express Kv1.3.[26] Compounds **5a**–**5c** were previously tested under the same assay conditions using L929 cells[9] and we include these data in Table 1 for comparison. Although we have found that there is a reasonable degree of coherence between the results from the two assays,[†] comparisons in the discussion are only made between data within a certain assay in the analysis of structure–activity relationships (Fig. 2).

When the α-methyl chalcone **11** was tested by electrophysiology, there was only a threefold decrease in potency relative to the unsubstituted chalcone **5b** (Table 1). We considered whether this could be explained by its structure, so the structures of both chalcone **5b** and a α-methyl chalcone (**16**), a conformational model of **11**, were determined by X-ray crystallography. The structure of **5b** (Fig. 3A) shows s-*cis* geometry of the enone as expected, with 20° torsion through the enone (O=CC=C), and *E*-arrangement of the olefin. There is a hydrogen bond between the enone carbonyl and *ortho*-hydroxy group on the benzofuran. On the other hand, **16** exhibits s-*trans* geometry about the enone, as anticipated, and the benzoic acid ring is twisted significantly from the plane of the benzofuran, with 130° torsion across the enone. We wondered whether it was

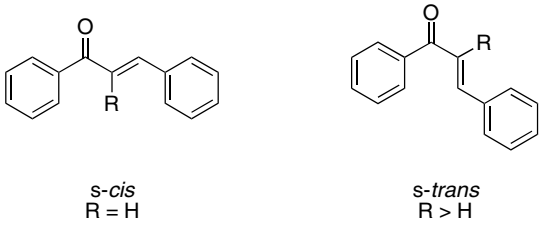


Figure 2. The conformational preference of chalcones switches from s-*cis* (left) to s-*trans* (right) if the α-substituent is bulkier than a hydrogen atom.

possible that **11** could adopt an s-*cis* form that could be responsible for its biological activity. However, and in agreement with the literature, molecular modelling indicated that the s-*cis* form in our α-methyl chalcones was higher in energy than the s-*trans* form, in the case of **11** by 7.4 kcal/mol, precluding the possibility of this being the biologically active conformation.[27] We then modelled **11** in the preferred s-*trans* conformation using the solid-state structure of **16** as a template and superimposed this onto the solid-state conformation of **5b**, using the two benzene rings as the bases for superimposition.[28] As can be seen from Fig. 3C, there is a remarkable degree of confluence in the spatial positioning of the two aromatic rings for s-*cis* and s-*trans* conformations. We propose that the olefinic portion of the enone may merely serve as a linker for the two aryl binding groups and that **11** could consequently still bind quite strongly to Kv1.3 in the s-*trans* conformation.

The chalcone **5c** was previously reported with an EC_{50} in L929 cells of 17 μM,[9] a 40-fold decrease in activity of the chalcone **5a** (Table 1), indicating that the 7-methoxy group is important for channel-blocking activity. As this group should have no effect on the conformation of the chalcone, this suggests that the methoxy group could favourably interact directly with Kv1.3. Truncations of the 4- and 6-positions are better tolerated in the chalcone series than removal of the 7-methoxy group. The compounds where the 6-hydroxy group was removed (**19** and **20**) both have EC_{50} values of 2.4 μM, and the 4-desmethoxy chalcone **26** has an EC_{50} of 1.7 μM (Table 1).

A crystal structure of the 6-deoxy chalcone **20** was obtained (Fig. 4A), and compared with the structure of chalcone **5b** (Fig. 4B). The enone system of **5b** is not coplanar, with 39° of torsion between the benzofuran and the methoxy-benzene ring (Fig. 4C). The crystal structure of compound **20** shows that even without the hydroxy group *ortho* to the carbonyl, the same conformation of the carbonyl is observed, and the torsion between the two ring systems is 30° (Fig. 4D). Overlay of the benzofuran rings of **5b** and **20** shows that the aryl rings are aligned similarly, and could occupy the same binding site in the ion channel (Fig. 4B).

We have determined that khellinone chalcone compounds can tolerate deletion of the 4-methoxy and 6-hydroxy groups with reasonable retention of Kv1.3-blocking activity. On the other hand, deletion of the

[†] Unpublished data.

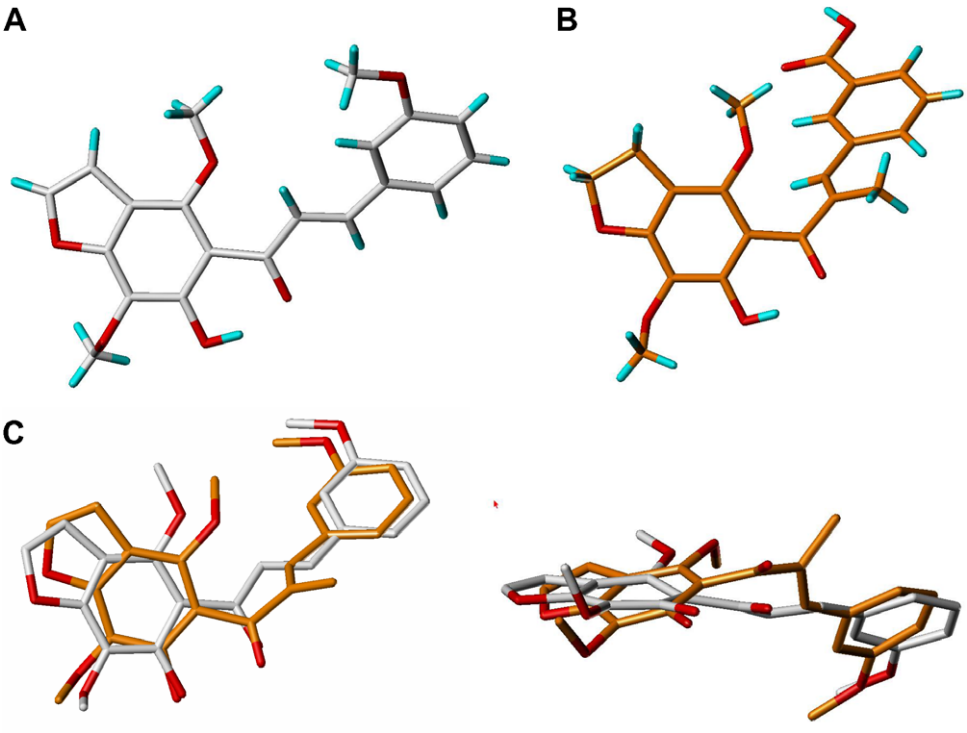


Figure 3. Crystal structure of (A) chalcone **5b**, (B) α-methyl chalcone **16**, and (C), two views of the proposed bioactive conformation of **11** (orange) superimposed on **5b** (white). For clarity, hydrogen atoms are not displayed.

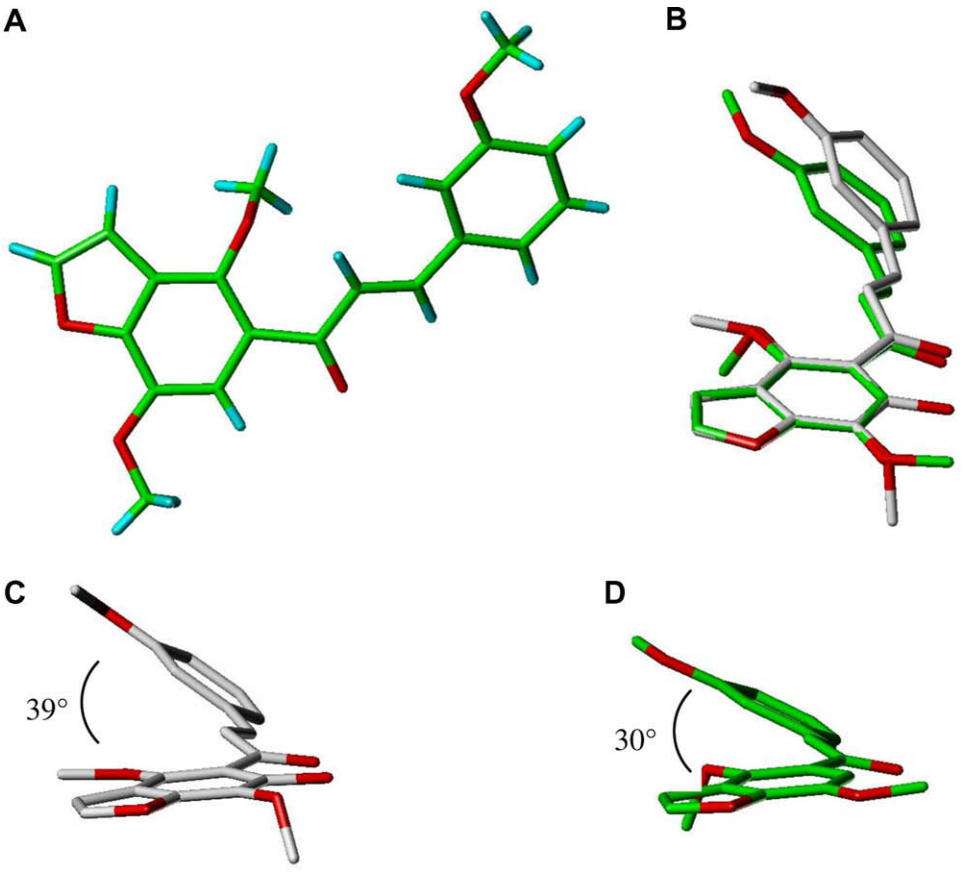


Figure 4. (A) Crystal structure of chalcone **20**, (B) alignment of the structures of **5** (white) and **20** (green). Structures showing torsion between benzofuran and benzene rings in chalcones **5** (C) and **20** (D).

7-methoxy group more significantly reduces potency. Since this alteration is unlikely to affect conformation, it is suggested that the 7-methoxy group in these compounds could directly and favourably interact with Kv1.3. Introduction of an α-methyl group into the enone, as in **11**, effected conformational change to the s-*trans* form, but without significant change in activity. Despite the observed conformational change, compounds **5b** and **11** show comparable alignment of their benzofuran and methoxyphenyl rings. The enone itself does not appear to be important for binding, but rather acts as a spacer to orient the key-binding elements.

Acknowledgments

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Supplementary data

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 674128-674130. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk]. ^{1}H NMR data for compounds **11**, **16**, **19**, **20** and **26** are available as Supporting Information. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.099.

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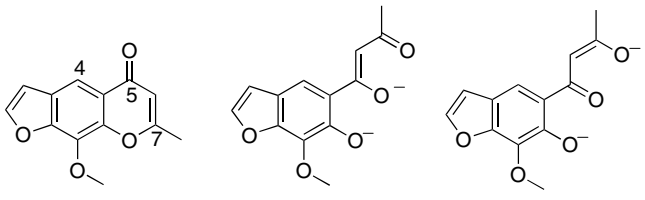
25. Carboxylic acid **25a** was the major product isolated and can be accounted for as follows. Alkaline hydrolysis of **24** to give the desired product **25b** would involve attack by hydroxide on C7 to give intermediate **24a**, followed by hydrolysis of the β-carbonyl with loss of acetate. Unexpected product **25a** could arise either via (a) hydrolysis of the carbonyl of tautomer **24b** with loss of acetone, or via (b) direct attack by hydroxide on C5 of **24** with loss of propyne. Attack of C5 in either **24** or **24b** would occur more readily than when a 4-methoxy substituent is present due to reduced steric hindrance and so can explain the different response to hydrolysis of **21** and **24**. We investigated the hydrolysis of a model system that involved isolation of its diketone intermediate prior to further hydrolysis (data not shown), the result of which suggests that only mechanism (a) accounts for the production of **25a**.



24 **24a** **24b**

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27. Minimisation was performed using the Tripos force-field, with Gasteiger-Huckel charges, using default values as implemented in sybyl7.3 (Tripos Associates, Missouri). This included the use of a distance-dependent dielectric of 1 using a non-bonded cutoff distance of 8 Å. The α-enone hydrogen atom in the solid-state structure of **5b** was changed to a methyl group. The resulting compound, the s-*cis* form of **11**, was allowed to minimize but force constants of 5 were applied to each of the four torsion angles in the enone in order to maintain the s-*cis* conformation. The same restraints were applied to the s-*trans* form of **11**, which was built in an identical conformation to the solid-state conformation of **16**. The s-*trans* form of **11** thus obtained was calculated to be 7.4 kcal/mol lower in energy to the s-*cis* form.
28. The above s-*trans* model of **11** was superimposed on the solid-state structure of **5b**, using the two benzene rings as the templates for superimposition, resulting in a 12 atom rmsd of 0.59 Å and which is shown in Figure 3C (note in Fig. 4 the mirror image of **5b** is represented relative to Fig. 3).