Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1051–1056

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

POTENT ANTIMITOTIC AND CELL GROWTH INHIBITORY PROPERTIES OF SUBSTITUTED CHALCONES

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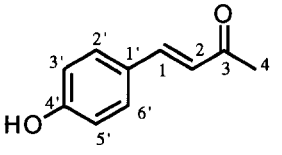
Received 10 February 1998; accepted 20 March 1998

Abstract: A series of substituted chalcones was synthesised and screened for cytotoxic activity against the K562 human leukaemia cell line. (*E*)-3-(3''-Hydroxy-4''-methoxyphenyl)-2-methyl-1-(3',4',5'-trimethoxyphenyl)-prop-2-en-1-one [IC_{50} (K562) 0.21 nM] was found to be the most active. A relationship between the conformation and cytotoxicity of the chalcones is discussed.

Keywords: antitumour compounds; chalcones; molecular modelling/mechanics; X-ray crystal structures.

Many clinically successful anticancer drugs are themselves either natural products or have been developed from naturally occurring lead compounds.[1] Great interest is currently being paid to drugs isolated from natural resources which have already been used as a medicine. The dried whole plant of *Scutellaria barbata* D. Don (*Labiatae*) is used in Traditional Chinese Medicine as an anti-inflammatory, an antitumour agent and a diuretic,[2] and the α,β-unsaturated ketone, (*E*)-1-(4′-hydroxyphenyl)but-1-en-3-one **1** has been isolated from this plant and found to possess moderate antitumour activity [IC_{50} (K562) 60 μM].[3] As part of a subsequent study to determine the features important for this anticancer we recently described the synthesis of various substituted phenylbutenones and the assays used to determine their antitumour activity in an *in vitro* cell culture system (MTT assay).[4] We now disclose the origin of the antitumour properties of the latest analogues, a series of α-methyl-substituted chalcones, based on the butenone **1**.



E-1-(4′-hydroxyphenyl)but-1-en-3-one **1**

The growth inhibitory activities of the chalcones described later were determined in the K562 human chronic myelogenous leukaemia cell line using the MTT assay. This assay is based on the reduction of the yellow coloured 3-(4',5'-dimethylthiazol-2'-yl)-2,5-diphenyl-tetrazolium bromide (MTT) by mitochondrial dehydrogenases of metabolically active cells to a purple-blue formazan, as detailed by Edmondson *et al.*[5] The IC_{50} concentration was calculated with reference to a standard curve constructed for control cells and represents the concentration which results in a 50% decrease in cell growth after five days incubation.

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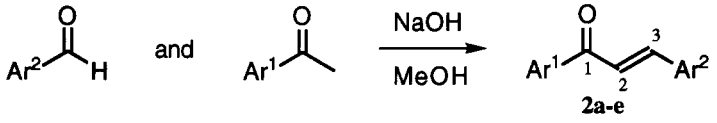
PII: S0960-894X(98)00162-0

When the methyl group of **1** was replaced with a substituted aryl group the resulting chalcone was often considerably active. The most active of a large number of chalcones[6] screened for cell growth inhibitory properties was the polymethoxylated chalcone **2a**. This chalcone **2a** was the subject of a recent patent application,[7] which prompts us to disclose our own findings in this area. Chalcone **2a** was prepared in excellent yield by the Claisen-Schmidt condensation of 3,4,5-trimethoxyacetophenone with isovanillin (scheme 1, table 1).[8] This method for the preparation of chalcones is particularly attractive since it specifically generates the (*E*)-isomer from substituted benzaldehydes and acetophenones, a large number of which are commercially available and inexpensive. Inspection of the ^1H nmr spectra clearly indicated that the chalcones **2** were both geometrically pure and were configured *trans* ($J_{H2\text{-}H3}$ 15-16 Hz).



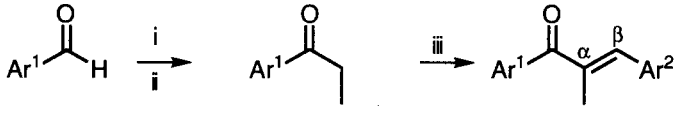
2a-e

Scheme 1

Table 1: Synthesis of *E*–chalcones **2a–d**

Ar^1	Ar^2	Chalcone	Yield (%)
3,4,5-trimethoxyphenyl	3-hydroxy-4-methoxyphenyl	**2a**	58
3,4,5-trimethoxyphenyl	3,4-[OCH_2O]phenyl	**2b**	85
3,4,5-trimethoxyphenyl	3,4-[OCH_2CH_2O]phenyl	**2c**	94
2,5-dimethoxyphenyl	4-(*N,N*-dimethylamino)phenyl	**2d**	78

Edwards and co-workers[9] have shown that the chalcone **5d**, possessing a methyl group on the α-carbon (C-2), is more cytotoxic than **2d**, which bears a hydrogen atom at the same site. No explanation for this effect was given. The chalcones **5a-d** were prepared by the piperidinium acetate catalysed aldol condensation of propiophenones **4a** and **4b** and the appropriate substituted benzaldehyde.[10] The substituted propiophenone **4a** and **4b**, (which are not commercially available), were prepared by addition of ethylmagnesium bromide to the appropriate benzaldehyde **3a** and **3b** followed by Swern or PCC oxidation of the intermediate secondary alcohols (scheme 2, table 2). Chalcones **5a-d** were obtained in modest yields and usually as a mixture of geometrical isomers (*E:Z ca.* 5:1), which fortunately were separable by column chromatography and crystallisation.



3a-b **4a-b** **5a-d**

3a and **4a** : Ar^1 = 3,4,5-trimethoxyphenyl
3b and **4b** : Ar^1 = 2,5-dimethoxyphenyl

Scheme 2

Reagents and Conditions: i. EtBr, Mg, THF, reflux, 1 h, then HCl; ii. DMSO, $(COCl)_2$, DCM, -78 °C, 2 h, then Et_3N (88% for **3a** → **4a**) or PDC, CH_2Cl_2, r.t., 2 d (44% for **3b** → **4b**); iii. Ar^2CHO, glacial AcOH, piperidine, dry EtOH, 4 Å molecular sieves, reflux, overnight.

Table 2: Synthesis of *E*–chalcones **5a–d**

Ar^1	Ar^2	Transformation	Chalcone	Yield (%)
3,4,5-trimethoxyphenyl	3-hydroxy-4-methoxyphenyl	**4a** → **5a**	**5a**	50
3,4,5-trimethoxyphenyl	3,4-[OCH_2O]phenyl	**4a** → **5b**	**5b**	35
3,4,5-trimethoxyphenyl	3,4-[OCH_2CH_2O]phenyl	**4a** → **5c**	**5c**	35
2,5-dimethoxyphenyl	4-(*N,N*-dimethylamino)phenyl	**4b** → **5d**	**5d**	20

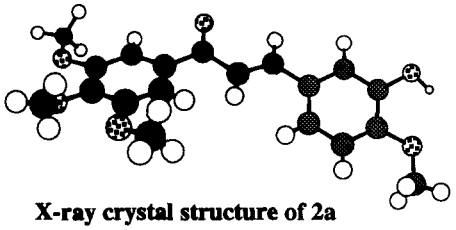
The chalcones **2a-d** and **5a-d** were screened for their ability to inhibit cell growth and the results are shown in table 3. It is clear that in all cases that addition of the methyl group to the carbon-2 atom greatly enhances the ability of the chalcone to inhibit cell growth. The chalcone **5d** MDL 27048, the most active of the analogues prepared by Edwards and co-workers[9] [ED (HeLa) 11.7 nM], was prepared and its activity compared to that of our own compounds. This known antimitotic compound, that acts by preventing the polymerisation of tubulin,[11,12] had an IC_{50} of 12 nM. It was a delight to find that the ability of chalcones **5a-c** to inhibit cell growth proved to be very high. Indeed the analogue **5a**, structurally similar to combretastatin-A-4,[13] showed an exceptionally impressive IC_{50} value of 0.21 nM. An investigation of the relationship between the carbon-carbon double bond geometry and cytotoxicity of chalcone **5a** revealed that the pure (*E*)-isomer was most active (IC_{50} 0.21 nM); a (*Z*)-enriched mixture (*E:Z*, 1:5, determined by ^1H NMR) gave an IC_{50} of 60 nM. The cytotoxicity (IC_{50}) of **5a** reported by Ikeda *et al.* was 0.62 nM for the $HeLaS_3$ cell line.[7]

Table 3: Cytotoxicities of *E*–chalcones **2a-d** and **5a–d** against the K562 cell line.

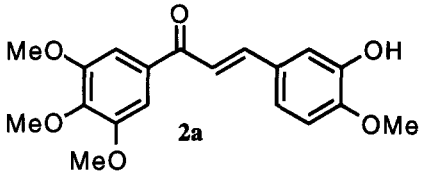
Ar^1	Ar^2	IC_{50} (nM)	IC_{50} (nM)
3,4,5-trimethoxyphenyl	3-hydroxy-4-methoxyphenyl	**2a** 4.3	**5a** 0.21
3,4,5-trimethoxyphenyl	3,4-[OCH_2O]phenyl	**2b** 300	**5b** 14
3,4,5-trimethoxyphenyl	3,4-[OCH_2CH_2O]phenyl	**2c** 80	**5c** 12
2,5-dimethoxyphenyl	4-(*N,N*-dimethylamino)phenyl	**2d** 36	**5d** 11

Single crystals of chalcones **2a** and **5a** were obtained by careful diffusion crystallization and their structures determined by X-ray crystal structure analysis (figure 1).[14] The crystal structure of **5a**, confirmed the (*E*)-configuration of the carbon-carbon double bond. A crystal of **5d** suitable for X-ray structure determination was also obtained; the structure of **5d** is reported here for the first time (figure 1). The X-ray crystal structures of **2a**, **5a**, and **5d** revealed an interesting relationship between the cytotoxicity of the chalcone and its conformation. The structures of the α-methyl-substituted chalcones **5a** and **5d** revealed that the carbon-oxygen and carbon-carbon double bonds are positioned *trans* relative to the C-1–C-2 single bond, as illustrated in the s-*trans* conformer (scheme 3). However, in the crystal structure of the chalcone **2a** the carbon-oxygen and carbon-carbon double bonds positioned *cis* relative to the C-1–C-2 carbon-carbon bond (the torsional angle O–C(1)–C(2)–C(3) of the enone system is -0.3°). The α-methyl chalcone **5a** adopts an s-*trans* conformation confirmed by a torsion angle O–C(1)–C(2)–C(3) of the enone system (180°)(scheme 3). This is certainly consistent with the known conformational preferences of chalcones; it is known that, for electronic reasons, enone systems prefer to adopt an s-*trans* conformation unless forced into the s-*cis* conformation by steric hindrance.[15] The X-ray crystal structures of many related chalcones possessing a hydrogen atom at C-2 (i.e. of type **2**) clearly illustrate the preference for the s-*cis* conformer (chalcone;[16] 4′-bromochalcone;[17] 4,4′-dimethylchalcone;[18] 4-chlorochalcone;[19] 4-methoxychalcone;[20] and 2′-hydroxy-2-methoxychalcone[21]). Several

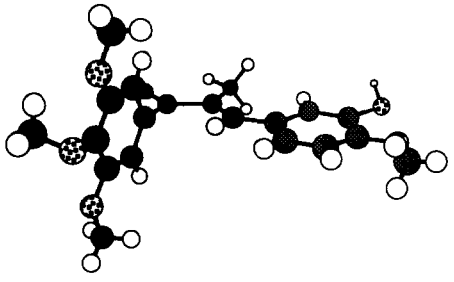
chalcones possessing a substituent at position 2 have been reported to adopt an s-*trans* conformation; e.g. 3- and 4-nitro-α-methoxychalcone;[22] α-azidochalcone;[23] 4′-bromo-4-dimethylamino-α-pyridinium-chalcone.[24]



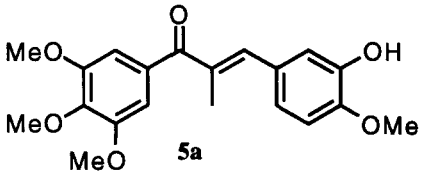
X-ray crystal structure of 2a



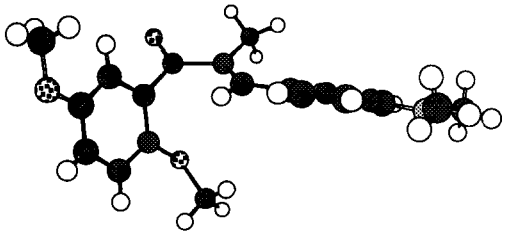
2a



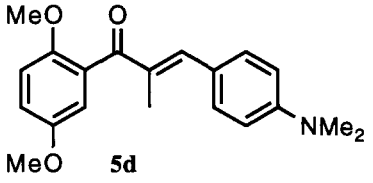
X-ray crystal structure of 5a



5a

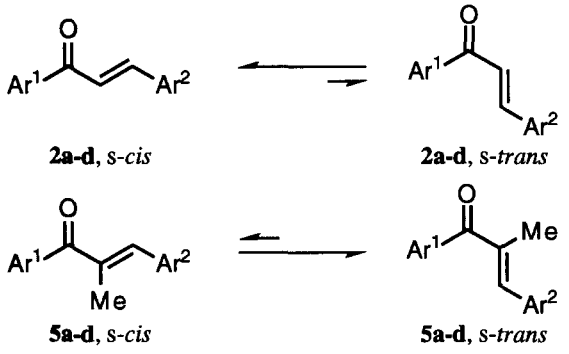


X-ray crystal structure of 5d



5d

Figure 1



2a-d, s-*cis* **2a-d**, s-*trans*

5a-d, s-*cis* **5a-d**, s-*trans*

Scheme 3

The difference in energies between the s-*cis* and s-*trans* conformers was investigated using molecular mechanic calculations. The modelling studies were performed using MacroModel 5.5 (MM2 force-field) on a Silicon Graphics Indigo work station. Full conformational searches were carried out by a Monte Carlo search using 1000 starting geometries. The calculations were performed on **2e** and **5e** ($Ar^1 = Ar^2 = Ph$) which

possess unsubstituted aryl groups. Eight minimum energy conformations were found for chalcone **2e**, most of which had the C=O and C=C bonds arranged s-*cis*. For **2e** the difference in energy (ΔE) between the lowest energy s-*cis* and s-*trans* conformers was 3.87 kJ mol^{-1}. For the α-methyl-substituted chalcone **5e**, all minimum energy conformers were s-*trans* and no s-*cis* conformation was found within 10 kJ mol^{-1} of the global energy minimum. This suggests that an s-*trans* conformation, among other things, is required for high activity. We believe that it is reasonable to use the unsubstituted chalcones **2e** and **5e** as models, since of the compounds **2** and **5**, most (**2a-c** and **5a-c**) have *ortho* hydrogen atoms on both aryl rings. These hydrogen atoms should not greatly effect the conformational preference of the enone unit.

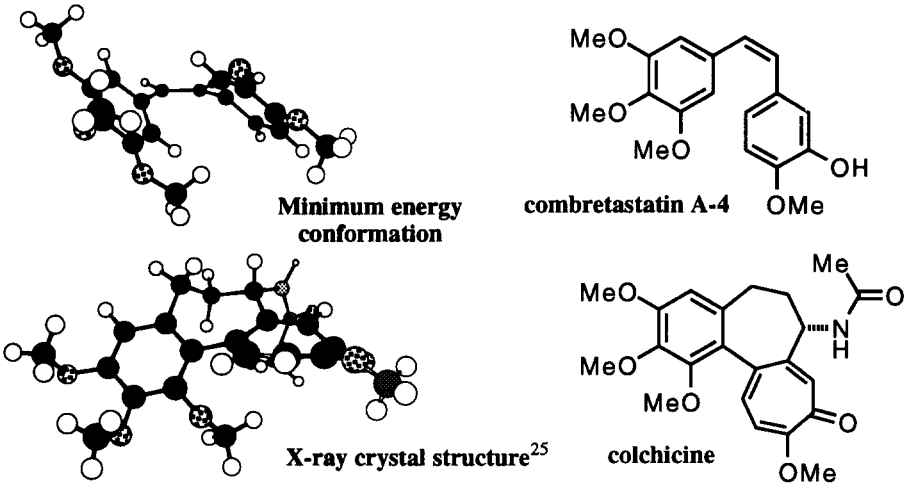


Figure 2

In chalcones **5a** and **5d**, the enone system is not coplanar with the phenyl ring Ar^1 as indicated by the torsion angle between C(*ortho*)-C(*ipso*)-C(1)-C(2), which is 88° for **5a** and 56° **5d**. The structural similarities between the chalcones, combretastatin A-4[25] and colchicine[26] are clear (figure 2); they all possess a 3,4,5-trimethoxyphenyl group and another similarly substituted aromatic ring. The spatial relationship between the two aromatic rings of combretastatin A-4 and colchicine and similar drugs has already been reported to be an important structural feature that determines their ability to bind to tubulin.[27] It therefore appears that the chalcone **5a** fits better into the colchicine binding site of tubulin than the chalcone **2a**, since it can adopt a conformation more similar to that of combretastatin A-4 and colchicine. The design of other antitumour agents, based on the above findings is currently underway; the results of synthetic and biological studies will be reported in due course.

Acknowledgements

We thank the Cancer Research Campaign and the Chemistry Department of UMIST for support of this work and Sally Haran and Tim Ward of the PICR Cell Culture Unit for maintaining the cell line, and providing valuable assistance during the cell growth inhibition experiments.

References and Notes

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