Evaluation of the radical scavenging activity of a series of synthetic hydroxychalcones towards the DPPH radical

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Abstract: Sixteen hydroxychalcones were synthesized in sufficient purity by the Claisen–Schmidt condensation between appropriate acetophenones and aromatic aldehydes. All the compounds were evaluated for their ability to scavenge the stable free 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Important structure–activity relationships were observed that strongly contribute to the knowledge for the design of DPPH radical scavenging chalcones. Relevant theoretical parameters were computed in an attempt to understand and explain the obtained experimental results.

Keywords: hydroxychalcones; 4'-chlorohydroxychalcones; synthesis; radical--scavenging activity; DPPH free radical.

INTRODUCTION

Chalcones (1,3-diarylprop-2-en-1-ones) are open-chain flavonoids consisting of two aromatic rings **A** and **B** that are joined by a three-carbon α,β-unsaturated carbonyl system. These compounds display a large number of biological activities, some of which are believed to correlate with their antioxidant potential.[1,2] For this reason, up-to-now many chalcones have been assessed[2] for their radical scavenging activity (*RSA*) towards various radicals. In this respect, the stable 2,2--diphenyl-1-picrylhydrazyl (DPPH) radical was the most employed target. The DPPH radical scavenging activity of chalcones was found to be favoured by the presence of the α,β-unsaturated carbonyl system,[3] and one or more phenolic groups, particularly arranged in either a pyrogallol or catechol moiety.[3–7] In addition, the introduction of an electron donating functionality, such as a prenyl or methoxy group, next to the phenolic group was regarded as important because these substituents increase the rate of hydrogen atom transfer from the phenolic

group.[6,8] However, the influence of the methoxy group on the RSA of hydroxy-chalcones was studied mainly in ring **A** because of the structural similarity of these compounds to many naturally-occurring chalcones.[1]

In the present study, a set of various hydroxychalcones possessing one or two phenolic groups in ring **B** and their analogues in which a methoxy substituent was inserted next to the phenolic group was synthesized. Ring **A** was unsubstituted or contained a *p*-chloro group. The RSA of all the chalcones was studied against the free DPPH radical using relevant theoretical parameters.

EXPERIMENTAL

General

The hydroxylated chalcones were synthesized in high yields (Table I) by variants of the Claisen–Schmidt condensation.[9,10] The melting points were obtained using a Mel-Temp 1102D-230 VAC instrument and are reported uncorrected. The radical scavenging activity of chalcones toward DPPH was determined on a Helios gamma UV–Vis spectrophotometer.

TABLE I. Substitution pattern, yields, melting points (m.p.), SC_{50}, bond dissociation enthalpy (*BDE*) and ionization potential (*IP*) values of the investigated chalcones **1–16**
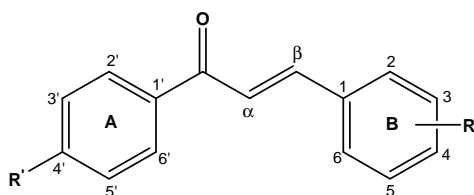
Compound	R'	R	Yield[a] %	M.p. °C	SC_{50}[b] mM	*BDE* kJ mol^{-1}	*IP* kJ mol^{-1}
1	H	2-OH	68	154–155	437.44	314.5	7.52
2	Cl	2-OH	73	143–145	465.60	316.6	7.58
3	H	3-OH	68	130–133	180.50	329.9	7.54
4	Cl	3-OH	78	106–109	181.25	330.8	7.62
5	H	4-OH	81	184–185	47.23	315.4	7.30
6	Cl	4-OH	83	175–176	111.29	317.1	7.38
7	H	3,4-OH	38	179–180	0.05	280.0	7.13
8	Cl	3,4-Di-OH	56	201–202	0.87	281.5	7.21
9	H	2-OH-3-OCH$_3$	69	112–113	12.19	312.4	7.19
10	Cl	2-OH-3-OCH$_3$	74	129–131	9.71	313.0	7.27
11	H	3-OH-4-OCH$_3$	72	92–94	264.38	317.9	7.05
12	Cl	3-OH-4-OCH$_3$	74	195–196	53.76	327.4	7.12
13	H	4-OH-3-OCH$_3$	70	91–95	158.74	314.4	7.01
14	Cl	4-OH-3-OCH$_3$	79	268–270	4.50	311.9	7.09
15	H	4-OH-3,5-di-OCH$_3$	80	powder	18.93	295.2	6.78
16	Cl	4-OH-3,5-di-OCH$_3$	83	119–121	2.31	296.1	6.86
Caffeic acid[c]	–	–	–	–	0.05	279.8	7.55

[a]Isolated yields; [b]the SC_{50} value refer to the chalcone concentration providing 50 % scavenging of DPPH radicals present in the test medium; [c]used as a positive control

The quantum chemical calculations were performed using the Gaussian 09 package[11] on a MADARA grid. The geometries of all possible conformational isomers of the studied compounds, radicals, radical cations were fully optimized by application of the UB3LYP functional in conjunction with the 6-31G* basis set. The optimized structures were further characterized by analytic computations of harmonic vibrational frequencies at the same level. Only the results for the most stable conformers according to the calculation were employed in the analysis of the structure–activity relationship.

DPPH radical scavenging assay

The radical-scavenging activity (RSA / %) of the chalcones **1**–**16** was determined using the DPPH radical in ethanol (0.1 mM), as described by Nenadis and Tsimidou.[12] Briefly, an aliquot (2960 µL) of a 0.1 mM ethanolic DPPH solution was mixed with 40 µL of each ethanolic sample solution to achieve concentrations of 0.5, 0.9, 1.8, 3.6 and 7.2 mM. The decrease of the light absorption at 516 nm of the DPPH radical solution was measured 20 min after addition of each chalcone sample. Caffeic acid was used as a positive control. The results were expressed as $RSA = (A(t = 0) - A(t = t')) \times 100/A(t = 0)$. The absorbance ($A$) values were corrected for radical decay using blank solutions. Each measurement was performed in triplicate at 25 °C. The SC_{50} value for each compound, representing its millimolar concentration providing 50 % scavenging of the DPPH radicals present in the test medium, was calculated from the $RSA = f$(concentration) curves. The standard deviations were below 10 %. The SC_{50} values were used in the structure–activity relationship study.

Statistical analysis

The correlation between the theoretical and experimental data was studied in terms of the correlation coefficient, *r*, also known as the Pearson product–moment correlation coefficient, which was computed using Excel software. The significance of the correlation was also calculated. The Ward method was used for clustering of the hydroxychalcones based on their radical scavenging ability (SC_{50}) and computed molecular descriptors. The aim was to find a theoretical parameter that discriminates between active and inactive chalcones.

RESULTS AND DISCUSSION

Sixteen chalcones with a hydroxycinnamoyl motif in their molecules were selected for evaluation of the RSA towards the DPPH radical. The chalcones possessed either a phenolic group or a combination of a phenolic and a methoxy group at various positions on the ring **B**, and had either a *p*-chloro group or no substituent on ring **A** (Table I). All the chalcones were obtained in sufficient purity by the Claisen–Schmidt condensation between acetophenone derivatives and the appropriate aromatic aldehyde (see Experimental).

The DPPH radical scavenging ability of all the chalcones was evaluated spectrophotometrically and compared to those of the well-known antioxidant caffeic acid (3,4-dihydroxycinnamic acid) (Table I). It was observed that the activity of chalcones having one or two phenolic groups in their ring **B** decreased in the following order depending on the position of the phenolic group: 3,4-di-OH (**7**) >> >> 4-OH (**5**) > 3-OH (**3**) > 2-OH (**1**). Insertion of a *p*-chloro group in ring **A** of these compounds did not affect their activity when 2-OH (**2**) and 3-OH (**4**) substituents were present in ring **B** but led to a 2- and 17-fold decrease in the *RSA* of

the chalcones with 4-OH (**6**) and 3,4-di-OH (**8**) groups, respectively. The two most active chalcones, **7** and **8** showed a RSA commensurable with that of caffeic acid, which was due to the presence of the 3,4-dihydroxycinnamoyl (catechol) moiety in their molecules. In particular, the electron-donating hydroxyl group at C-3 in the phenyl ring of caffeic acid is known[13] to increase the rate of hydrogen atom transfer from the phenolic group at C-4 to the DPPH radicals, resulting in the formation of an *o*-hydroxyl phenoxyl radical. This radical is more stable due to the delocalization of the unpaired electron across the entire molecule and the intramolecular hydrogen bonding interaction. The *o*-hydroxyl radical is also easier to oxidize further to the final product *o*-quinone.[14] Methoxylation of one of the hydroxyl groups of 3,4-dihydroxychalcone **7** to form 3-hydroxy-4--methoxy- (**11**) and 4-hydroxy-3-methoxychalcones (**13**) led to a great loss in activity because the chalcones **11** and **13** cannot form quinone oxidation products. The presence of a methoxy group was favourable when it was next to the 2-OH group (compound **9**) and when two methoxy groups were inserted from both sides of 4-OH group (compound **15**). It was also found that insertion of chlorine at the *p*-position in ring **A** enhanced the *RSA* of all chalcones substituted with a combination of phenolic and methoxy groups and these chalcones were more active than the chlorinated chalcones having hydroxyl groups only.

In order to understand the experimental results, the structure of the studied molecules and their radicals were studied using the Density functional theory (DFT) employing the UB3LYP functional and the 6-311G* basis set. It was taken into consideration that the two generally accepted mechanisms[15] for the antioxidant action of phenolic compounds (ArOH) are hydrogen atom transfer (HAT, Eq. (1)) and single-electron transfer followed by proton transfer (SET–PT, Eqs. (2) and (3)).

$$ArOH \rightarrow ArO^{\bullet} + H\cdot \tag{1}$$
$$ArOH \rightarrow ArOH^{+\bullet} + e^{-} \tag{2}$$
$$ArOH^{+\bullet} \rightarrow ArO^{\bullet} + H^{+} \tag{3}$$

The corresponding reaction enthalpies, bond dissociation enthalpy (*BDE*, related to Eq. (1)) and the ionization potential (*IP*, related to Eq. (2)), were calculated (Table I) and used as theoretical parameters to elucidate the substituent effect on the antioxidant activity. While the *IP* values were found not to discriminate between the active and inactive compounds, a moderate but statistically significant positive correlation ($r = 0.47$, $p < 0.05$) was obtained between the *BDE* and SC_{50} values. A hierarchical cluster analysis of these data yielded a dendrogram with horizontal lines representing the chalcones and vertical lines showing the similarity between pairs of chalcones based on their SC_{50} and *BDE* values (Fig. 1). Two main clusters were observed showing that in fact the *BDE* discriminated between the active ($SC_{50} < 2.31$ mM) and the inactive chalcones ($SC_{50} >$

> 4.50 mM). The *BDE* values calculated for the active compounds were all below 300 kJ mol^{-1} (Table I). However, an outlier was present in the cluster of the active chalcones, namely compound **15**, which had a *BDE* value of 295.2 kJ mol^{-1} but did not show RSA (Table I, Fig. 1). The reason for this phenomenon remains unclear.
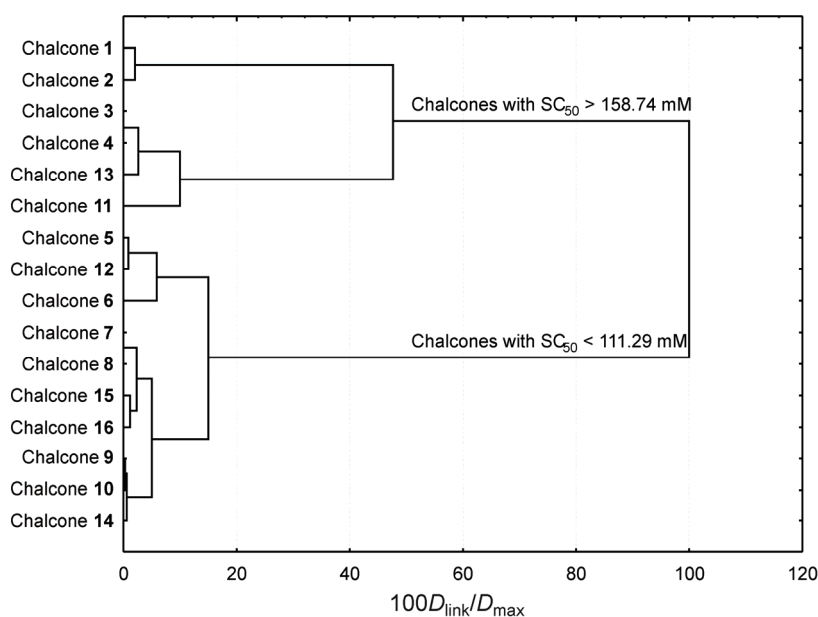

Fig. 1. Hierarchical dendrogram obtained by the method of complete linkage. The dendrogram shows that the bond dissociation enthalpy values discriminate between the active and inactive chalcones (D_{link} – linkage distance; D_{max} – maximal linkage distance.

With the BDE values in mind, another observation was made indicating that the electronic effects of *p*-chloro group in ring A did not influence the RSA of chalcones. This non-influential behaviour of the *p*-chloro group was in agreement with the results reported by Kim *et al*.[5] However, in our study, it was found that the *p*-chloro group did not affect the RSA only of the chalcones having *o*-OH and *m*-OH functionalities in ring B. Insertion of *p*-chloro group in ring A of the chalcones with *p*-OH and 3,4-di-OH groups decreased their RSA. When attached to the chalcones having a combination of hydroxyl and methoxy groups in ring B it increased their activities. The course of action of the *p*-chloro group however remains unclear.

An attempt was also made to correlate the SC_{50} results of the chalcones with some other molecular descriptors. However, the parameters refractivity, polarizability, mass, coefficient of molecular partition octanol–water (log *P*), dipole

moments and HOMO and LUMO energies were disregarded due to their inability to differentiate the active compounds (data not given).

The observed relationships between the position and the surrounding of the phenolic groups in ring **B** and the *RSA* of hydroxychalcones may be useful for the design of chalcone-like compounds with radical scavenging abilities.

CONCLUSIONS

A series of hydroxychalcones was synthesized and evaluated for their DPPH radical scavenging activity. Depending on the position of the phenolic group in ring **B**, the radical scavenging activity of the hydroxychalcones decreased in the following order: 3,4-di-OH >> 4-OH > 3-OH > 2-OH. The presence of a methoxy group was favourable when it was next to the 2-OH group and when two methoxy groups were inserted on both sides of the 4-OH group. Insertion of a 4'--chloro group in ring **A** enhanced the activity of all chalcones substituted with a combination of phenolic and methoxy groups and these compounds were more active than 4'-chloro chalcones having hydroxyl groups only. The reported structure–activity observations strongly contribute to the knowledge for design of DPPH radical scavenging chalcones.

И З В О Д

ИСПИТИВАЊЕ СПОСОБНОСТИ ХВАТАЊА DPPH РАДИКАЛА
СЕРИЈЕ СИНТЕТИЧКИХ ХИДРОКСИХАЛКОНА

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Синтетисано је шеснаест хидроксихалкона Клајзен–Шмитовом кондензацијом, полазе-ћи од одговарајућих ацетофенона и ароматичних алдехида. Свим једињењима је испитана способност хватања стабилних слободних радикала 2,2-дифенил-1-пикрилхидразила (DPPH). Утврђени су односи структура–активност који омогућавају дизајн нових халкона као хватача DPPH радикала. Израчунати су релевантни теоријски параметри у циљу разумевања и објашњавања експерименталних резултата.

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