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Structural Bases for the Inhibition of Aldose Reductase by Phenolic Compounds

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Abstract—Aldose reductase (ALR2) is an enzyme involved in the development of long-term diabetic complications. In the search for aldose reductase inhibitors less acidic than carboxylic acids, phenolic compounds related to benzopyran-4-one and chalcone are particularly interesting because they possess good inhibitory properties. In order to investigate the similarities between these two classes of compounds and to provide a structural basis for their inhibition of ALR2, the existing structure–activity relationships were reconsidered. To this end, the acidity constants of a set of chalcones were measured and compared with those of benzopyran-4-one derivatives. Then, having established the relevant protonation state of these phenolics at physiological pH, a conformational analysis was performed on the most active benzopyran-4-one and chalcone derivatives and the results were compared with the crystal structures of some analogues. Finally, molecular docking of the most active chalcone into the ALR2 binding site was performed, and the structure of the enzyme–inhibitor complex was compared with that of the complex formed between ALR2 and a previously-obtained benzopyran-4-one derivative.

Introduction

Aldose reductase (Alditol:NADP$^+$ oxidoreductase, E.C. 1.1.1.21, ALR2) is the first enzyme of the polyol pathway; glucose flux through this pathway, during diabetes, has been linked to the development of long-term diabetic complications. Thus, ALR2 inhibitors (ARIs) have been developed as potential agents to prevent or delay the onset of diabetic complications.[1]

To date, carboxylic acid and cyclic imide derivatives are among the most active and well-known inhibitors of ALR2.[2] Structure–activity relationships within these two classes of compounds have revealed that the presence of an acidic function (carboxylate or cyclic imide) is very important for the inhibition of ALR2. In the crystal structures of ALR2-inhibitor complexes, the negatively charged carboxylates or cyclic imides hydrogen bond to three key enzyme residues, Tyr48, His110 and Trp111, and give strong electrostatic interactions with the positively charged nicotinamide ring of NADP$^+$.[2]

Although an acidic function is important for the inhibition of the enzyme, the development of new ARIs having better pharmacokinetic properties is subject to the pK_a of the inhibitors not being too acidic. Very recently, we modified the aglycone ARI Quercetin, a natural flavonoid, and discovered a new, more potent and selective ARI (7-hydroxy-2-(4′-hydroxybenzyl)-4*H*-1-benzopyran-4-one, **1**, IC_{50}: 2.50 μM).[3] The 7-hydroxyl substituent, which was identified as being fundamental for the inhibition of ALR2, has a pK_a value of about 7.3 and is, therefore, considerably less acidic than carboxylic acids. SAR analysis led us to conclude that 7-hydroxy-4*H*-1-benzopyran-4-one derivatives bind ALR2 in their dissociated, anionic form. Interestingly, docking and molecular modeling simulations[3] suggested that the dissociated 7-hydroxyl hydrogen bonds to Tyr48 and His110 (Fig. 1), thus providing a good structural replacement for carboxylates. The 2-benzyl substituent, owing to its hydrophobic aromatic nature and particular conformation, was found to fit optimally an additional hydrophobic pocket of the enzyme, lined by Trp111 and Leu300, which has been well documented in the literature[3–5] Binding of aromatic substituents into this additional pocket provides active and selective inhibitors with respect to the closely related enzyme aldehyde reductase.[3–5] Finally, in the structure of the complex, the 4′-hydroxy group hydrogen bonds to Thr113 (Fig. 1).

Hydroxylated chalcones are interesting phenolic compounds possessing ALR2 inhibitory activity.[6–8] Of the

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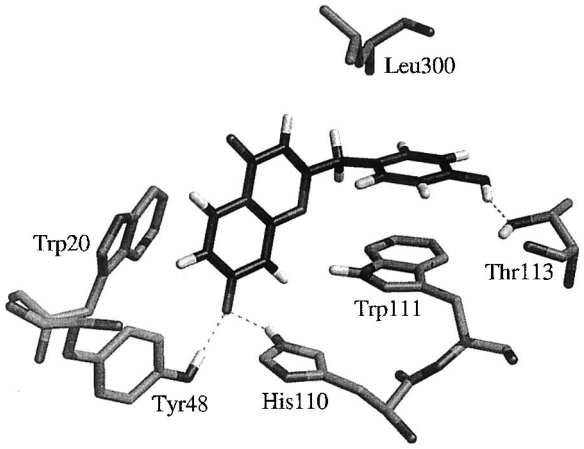
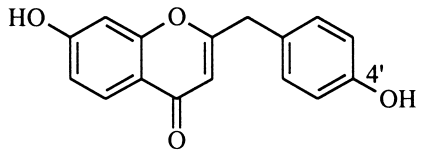
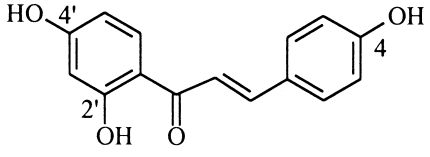


Figure 1. Enzyme residues interacting with the benzopyran-4-one derivative **1** in the structure of the enzyme-inhibitor complex.

compounds studied, 2′,4,4′-trihydroxychalcone (**2**) was one of the most active derivatives of the class (IC_{50}: 7.60 μM[8]). Again, in the chalcone set of compounds, one hydroxy group turned out to be very important for the inhibition of the enzyme, namely, the hydroxyl at position 4′.[6–8] Therefore, hydroxylated benzopyran-4-one and hydroxylated chalcone derivatives might share similar structure–activity relationships.



(1)



(2)

To examine the possible similarities between these two classes of ARIs, the acidity constants (pK_a) of four chalconic derivatives (**4**–**7**) were first measured and compared with those of benzopyran-4-one derivatives.[3] Then, having established the relevant protonation state of hydroxychalcones at physiological pH, a complete conformational analysis of **1** and **2** was performed using theoretical simulations, both in vacuo (PM3) and in water (PM3/SM3). The most stable conformers were then compared with the crystal structure of 7-methoxy-2-(4′-hydroxybenzyl)-4*H*-1-benzopyran-4-one (**8**), determined in the present work, and of 2′,4′-dihydroxy-3-methoxychalcone.[7] Finally, docking of **2** into the ALR2 binding site and energy minimization of the complex using molecular mechanics allowed us to show how 4′-hydroxychalcones interact with the enzyme and to compare this with the binding mode of the benzopyran-4-one derivative **1** previously investigated.

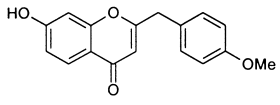
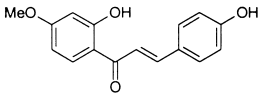
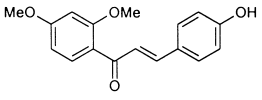
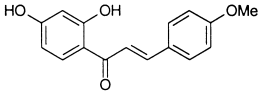
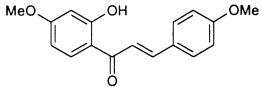
Results and Discussion

Acidity constants

The pK_a values of a set of chalcone derivatives were determined spectrophotometrically, and are reported in Table 1. The pK_a value of the chalcone derivative **6** is 7.47, and is, therefore, very close to the pK_a of the benzopyran-4-one derivative **3** (7.35, Table 1). At variance with compound **6**, which has a free hydroxyl at position 4′, compound **4** has a 4′-methoxyl and cannot dissociate in this position. The pK_a of the chalcone **4** is about one unit higher (8.44), thus resulting in a less acidic compound. To exclude the possibility that dissociation of **6** occurs at the 2′-hydroxyl instead of at the 4′-hydroxyl, the pK_a of the derivative in which only the 2′-hydroxyl was maintained (**7**) was measured and found to be much less acidic (10.05, Table 1). Likewise, the pK_a of derivative 5 (8.52), in which dissociation can occur only at the 4-hydroxyl, is about one unit higher than the pK_a of **6**. Therefore, the hydroxyl at position 4′ is the more acidic hydroxyl in these chalcones.

Structure–activity relationships inferred from previous work[3,6–8] and the present pK_a determinations indicate that **1** and **2** exert their inhibitory activity towards ALR2 in their anionic, dissociated forms, the most active form of benzopyran-4-one derivatives being dissociated at the 7-hydroxyl, and that of chalcones being dissociated at the 4′-hydroxyl. For this reason, the chalcone derivative **2** will be considered as dissociated at

Table 1. pK_a values and inhibitory activities toward ALR2

Compound		pK_a (+/−S.D.)	ALR2 IC_{50} [b]
3		7.35 (+/−0.07)[a]	7.01[a]
4		8.44 (+/−0.18)	26% inh. (27.01 μM)[c]
5		8.52 (+/−0.06)	47.55[c]
6		7.47 (+/−0.08)	27.60[d]
7		10.05 (+/−0.09)	40% inh. (78.54 μM)[d]

[a]From ref 3.
[b]IC_{50} values (μM) (concentration of the inhibitor required to produce 50% inhibition of the enzyme catalyzed reaction) or percent inhibition at a given concentration.
[c]From ref 8.
[d]From ref 7.

position 4′ in the conformational analysis, docking and molecular mechanics calculations presented below.

Crystal structure of 7-methoxy-2-(4′-hydroxybenzyl)-4*H*-1-benzopyran-4-one (8)

The asymmetric unit of 7-methoxy-2-(4′-hydroxybenzyl)-4*H*-1-benzopyran-4-one (**8**) contains two crystallographically independent molecules, illustrated in Figure 2, along with the atom-labelling scheme used throughout.

There is very good agreement between the corresponding bond distances and angles of the two molecules, and their values compare well with those reported for parent molecules.[9–12] According to previous reports,[9–2] both conformers are planar within experimental uncertainty, the maximum deviation from mean least-squares planes being 0.037 (3) and 0.035 (2) Å, respectively. The methoxy groups are nearly coplanar with the aromatic rings to which they are bonded, as previously found for parent derivatives.[11] Nevertheless, their orientations are slightly different, with torsional angles about the C–O bonds of 6.5 (4) and −3.0 (5)°, respectively, in the two independent molecules. The two molecules differ basically in the orientation of the 4′-hydroxybenzyl substituent with respect to the benzopyran-4-one moiety. The O1-C1-C11-C12 torsional angle is −4.4 (6)° in molecule A, and 50.4 (5)° in the B-labelled one (see Fig. 2). Thus, the loss of crystallographic equivalence between the molecules represents a case of coexistence (in the same crystal) of two conformational isomers. Other relevant conformational differences involve the torsional angles about the C11-C12 bonds, but, in both molecules, the phenyl rings are nearly perpendicular with respect to the benzopyran-4-one mean planes. The involved dihedral angles are 89.5 (8) and 86.29 (8)°, respectively.

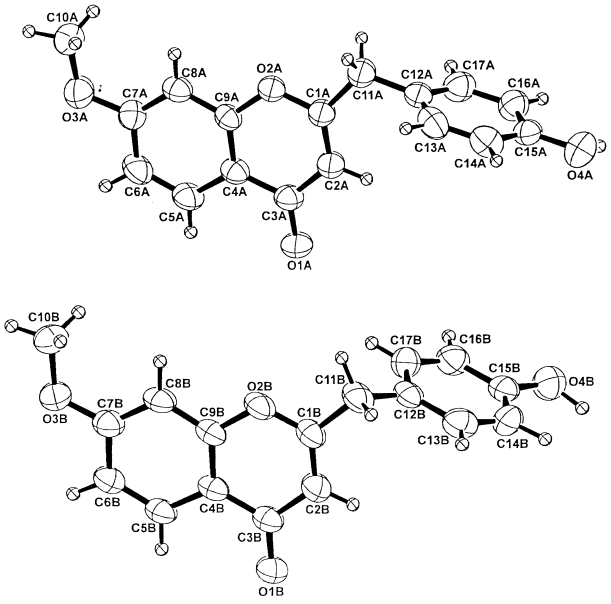


Figure 2. X-ray molecular structure of **8** with atom numbering scheme. Thermal parameters enclose 50% probability.

It is of interest to note that both molecules, in spite of strong conformational differences, pack in the crystal as hydrogen-bonded dimers. Each conformer is linked to a centrosymmetrically related molecule through two strong hydrogen bond interactions (see Fig. 3). In both cases, such an interaction involves the hexocyclic pyrone oxygen as hydrogen bond acceptor and the -OH function as donor. The O···O separations are 2.673 (4) and 2.682 (3) Å, and the subtended O-H···O angles are close to 180°. These interactions seem to play an important role in determining not only the crystal packing but also the molecular conformations.

Conformational analysis

Conformational analysis was performed by rotating the dihedral angles of **1** and **2** in steps of 20°, with energy minimization at each step. In some cases, the step was reduced to 10° or 5°. The results are reported in Tables 2 and 3.

The benzopyran-4-one derivative **1** possess two rotatable bonds, ϑ_1 and ϑ_2 (Table 2). The energy profiles are graphically displayed in Figure 4. Both in vacuo and in water, two symmetrically equivalent minima were detected for ϑ_1, one at 90° and the other at 270°. In these minima, the phenolic ring is perpendicular to the benzopyran-4-one ring. In vacuo, barriers of about 2.5 and 1 kcal/mol separate these minima. In water, the barriers are only slightly higher. Owing to the relatively low barriers, there is substantial conformational freedom around ϑ_1. As for ϑ_2, two shallow and symmetrically equivalent minima were located at 100–140° and 220–280°, with a very low barrier in vacuo. In water the barrier rises to only 1.7 kcal/mol, and the minima become better defined at 90° and 280° (Fig. 4).

The conformers of **1** compare quite well with the crystal structure of 7-methoxy-2-(4′-hydroxybenzyl)-4*H*-1-benzopyran-4-one (**8**). The benzopyran-4-one and the phenol rings are planar. While conformational analysis gave two equivalent minima at $\vartheta_1 = 90$ and 270°, the two crystallographically independent molecules in the crystal structure of **7** have angles of −4.4 and 50.4°. Even if the monomer having $\vartheta_1 = 50.4°$ approaches the calculated 90° conformer, the differences must be due to the finding that the benzopyran-4-one derivative in question packs in the crystal as a dimer. Since conformational barriers are relatively low, rotation around ϑ_1 in favour of a tight packing in the crystal is highly favoured. As

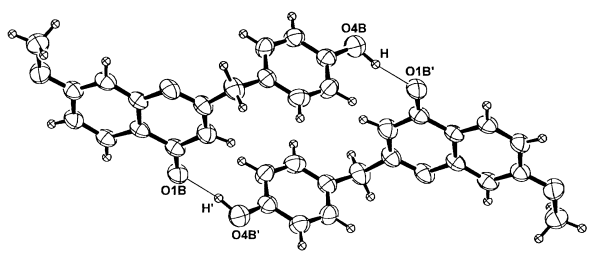
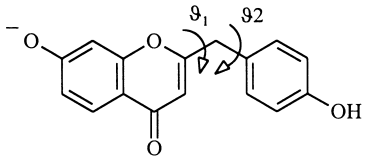


Figure 3. Hydrogen bonds (still lines) between centrosymmetrically related molecules of (**8**).

Table 2. Conformational analysis results for the 7-hydroxy-2-(4′-hydroxybenzyl)-4*H*-1-benzopyran-4-one derivative **1** both in vacuo (PM3) and in water (PM3/SM3)



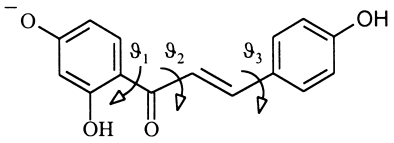
ϑ_1	ΔE^{PM3}	$\Delta E^{PM3/SM3}$	ϑ_2	ΔE^{PM3}	$\Delta E^{PM3/SM3}$
0	0.94	1.33	0	0.97	2.13
20	0.83	1.18	20	0.90	2.06
40	0.58	1.00	40	0.69	1.69
60	0.35	0.78	60	0.48	1.03
80	0.07	0.55	80	0.28	0.47
90	0.00	0.00	100	0.12	0.20
100	0.12	0.16	120	0.12	0.36
120	0.69	1.59	140	0.12	0.80
140	1.45	2.27	160	0.26	1.31
160	2.14	2.89	180	0.36	1.67
180	2.44	3.18	200	0.27	1.59
200	2.14	2.89	220	0.12	1.27
220	1.45	2.28	240	0.02	0.69
240	0.68	1.59	260	0.00	0.25
260	0.14	0.16	280	0.07	0.00
270	0.00	0.00	300	0.30	0.47
280	0.08	0.55	320	0.50	1.02
300	0.35	0.80	340	0.77	1.70
320	0.59	1.00	—	—	—
340	0.83	1.18	—	—	—

for ϑ_2, both the present calculations and the X-ray crystal structure agree that the phenyl ring is nearly perpendicular to the benzopyran-4-one mean plane.

The minimum-energy conformation of **1** is very similar to the conformation adopted when binding aldose reductase (Fig. 1). In the structure of the complex, $\vartheta_1 = 105°$ and $\vartheta_2 = 162°$ Therefore, **1** binds the enzyme with minimal changes of conformation. In the complex, the oxygen of the dissociated 7-hydroxyl hydrogen bonds to Tyr48 and His110 and the aromatic ring of the benzyl substituent is properly folded to be inserted into the additional hydrophobic pocket, being perpendicular with benzopyran-4-one.

The chalcone derivative **2** possesses three rotatable bonds, ϑ_1, ϑ_2 and ϑ_3 (Table 3). Conformational analysis around ϑ_1 provided two minima, one at $\vartheta_1 = 180°$ and the other at $\vartheta_1 = 0°$ (Fig. 5). In both conformers, the phenyl ring is coplanar with the adjacent carbonyl group of chalcone. The minimum corresponding to $\vartheta_1 = 180°$ is 7.5 kcal/mol more stable than that at $\vartheta_1 = 0°$ both in vacuo and in water. This difference is probably due to the formation of an intramolecular hydrogen bond between the 2′-hydroxyl and the carbonyl oxygen, which is possible when $\vartheta_1 = 180°$ but not when $\vartheta_1 = 0°$ (because the 2′-hydroxyl is rotated in the opposite direction with respect to carbonyl). The conformations in which the phenyl and the carbonyl are perpendicular (90° and 270°) are highly unstable, because of a lack of conjugation between these two groups. Conjugation occurs between the negatively charged hydroxylate at

Table 3. Conformational analysis results for the chalchone derivative **2** both in vacuo (PM3) and in water (PM3/SM3)



ϑ_1	ΔE^{PM3}	$\Delta E^{PM3/SM3}$	ϑ_2	ΔE^{PM3}	$\Delta E^{PM3/SM3}$	ϑ_3[a]	ΔE^{PM3}	$\Delta E^{PM3/SM3}$
0	7.41	7.47	0	4.25	4.10	0	0.00	0.00
20	7.42	7.26	20	3.09	3.19	20	0.28	0.41
40	8.45	7.40	40	2.35	2.54	40	0.35	0.74
60	9.56	7.22	60	1.36	1.75	60	0.78	1.36
80	11.57	7.59	70	1.20	1.60	80	1.27	1.96
90	11.77	7.83	80	1.12	1.54	100	1.41	2.08
100	11.13	7.59	90	1.04	1.45	120	1.13	1.65
120	8.61	6.40	100	0.95	1.36	140	0.72	1.01
140	4.35	3.78	105	0.91	1.31	160	0.55	0.59
160	0.98	0.82	110	0.88	1.26	180	0.17	0.14
180	0.00	0.00	115	0.86	1.23	200	0.48	0.56
200	0.94	0.79	120	0.88	1.22	220	0.57	0.90
220	3.99	3.48	125	0.88	1.21	240	1.00	1.55
240	8.61	6.39	130	0.90	1.18	260	1.46	2.11
260	11.10	7.58	140	0.47	0.58	280	1.53	2.16
270	11.78	7.81	160	0.06	0.09	300	1.20	1.66
280	11.58	7.58	180	0.00	0.00	320	0.70	0.99
300	9.59	7.24	200	0.06	0.09	340	0.47	0.51
320	8.45	7.42	220	0.47	0.58	—	—	—
340	7.40	7.26	240	0.87	1.22	—	—	—
—	—	—	245	0.86	1.23	—	—	—
—	—	—	250	0.88	1.26	—	—	—
—	—	—	260	0.95	1.35	—	—	—
—	—	—	280	1.12	1.54	—	—	—
—	—	—	300	1.36	1.75	—	—	—
—	—	—	320	2.35	2.53	—	—	—
—	—	—	340	3.08	3.19	—	—	—

[a]Conformational analysis for ϑ_3 was performed starting from the $\vartheta_2 = 115°$ conformer (second conformer of **2**).

position 4′ and the carbonyl, and is probably the reason why chalcones are more acidic than the hydroxyl of phenol. The same must be true for the 7-hydroxyl of **1**, the only difference with chalcone being that conjugation between the negative hydroxylate and the carbonyl occurs within the closed ring of benzopyran-4-one.

Conformational analysis around ϑ_2 gave results particularly relevant in the light of the conformation apt to bind aldose reductase. Three minima were detected, the most stable being at $\vartheta_2 = 180°$; the other two (symmetrically equivalent) were at 115 and 245° and only 0.86 kcal/mol less stable in vacuo and 1.23 kcal/mol less stable in water (Fig. 5). Even if the energy profile is very shallow in this region, further geometry optimization without any constraint and using increased convergence criteria confirmed that $\vartheta_2 = 115$ and 245° are true minima. In the more stable conformation ($\vartheta_2 = 180°$) chalcone is completely planar, in agreement with the crystal structure of 2′,4′-dihydroxy-3-methoxychalcone.[7] However, this conformation is not particularly suited to binding aldose reductase. In fact, provided that the negatively charged oxygen at position 4′ is properly aligned to give hydrogen bonds with Tyr48 and His110, the phenol substituent cannot fit the additional hydrophobic pocket, since it is coplanar with the rest of the

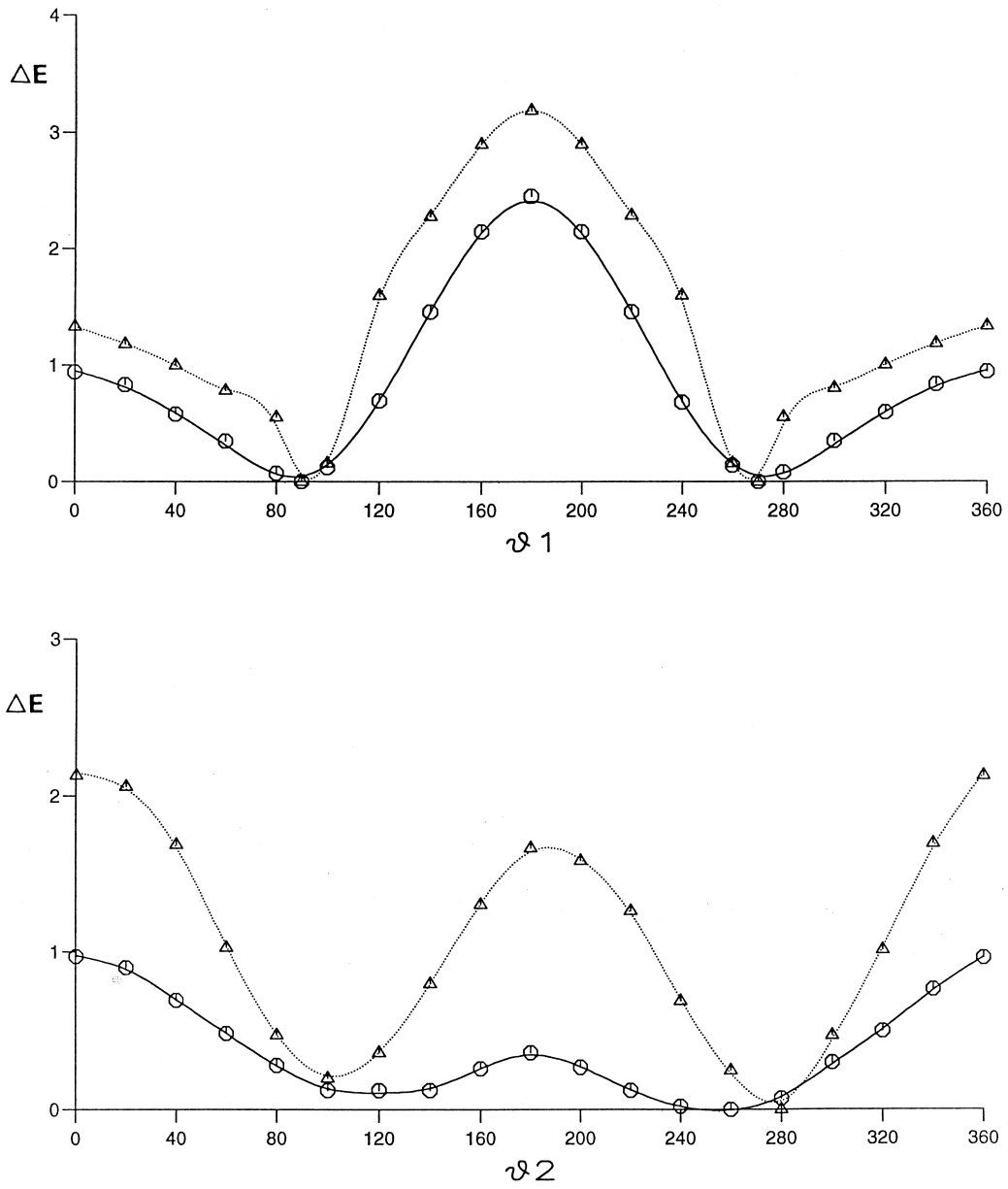


Figure 4. Energy profiles (kcal/mol) for the rotation around ϑ_1 and ϑ_2 of the benzopyran-4-one derivative **1** in vacuo (circles) and in water (triangles).

molecule. On the other hand, the conformer showing $\vartheta_2 = 115°$ is able to fit both pockets of the enzyme, since the phenyl ring carrying the negative hydroxylate and the phenolic ring are almost perpendicular. On closer inspection, an angle of $\vartheta_2 = 115°$ for chalcone closely resembles the $\vartheta_1 = 105°$ angle adopted by the benzopyran-4-one derivative 1 when binding the enzyme, and the two structures can be very well superimposed.

Finally, rotation around ϑ_3 of chalcone identified two equivalent minima at 180° and 0°, in which the phenolic ring is coplanar with the adjacent ethylene double bond. These minima are separated by rather low barriers (about 1.5 kcal/mol in vacuo and 2 kcal/mol in water) (Fig. 5).

Docking into the ALR2 binding site

The conformation of the chalcone **2** found to fit the aldose reductase binding site was docked into the enzyme, and the structure of the enzyme–inhibitor complex was refined with energy minimization using molecular mechanics methods, as previously described for benzopyran-4-one derivatives.[3]

The structure of the complex, with only a few important residues interacting with chalcone shown, is graphically reported in Figure 6. The dissociated hydroxyl at position 4′ hydrogen bonds to Tyr48 and His110, and the phenolic ring binds the additional hydrophobic pocked lined by Trp111 and Leu300. The 4-hydroxyl hydrogen

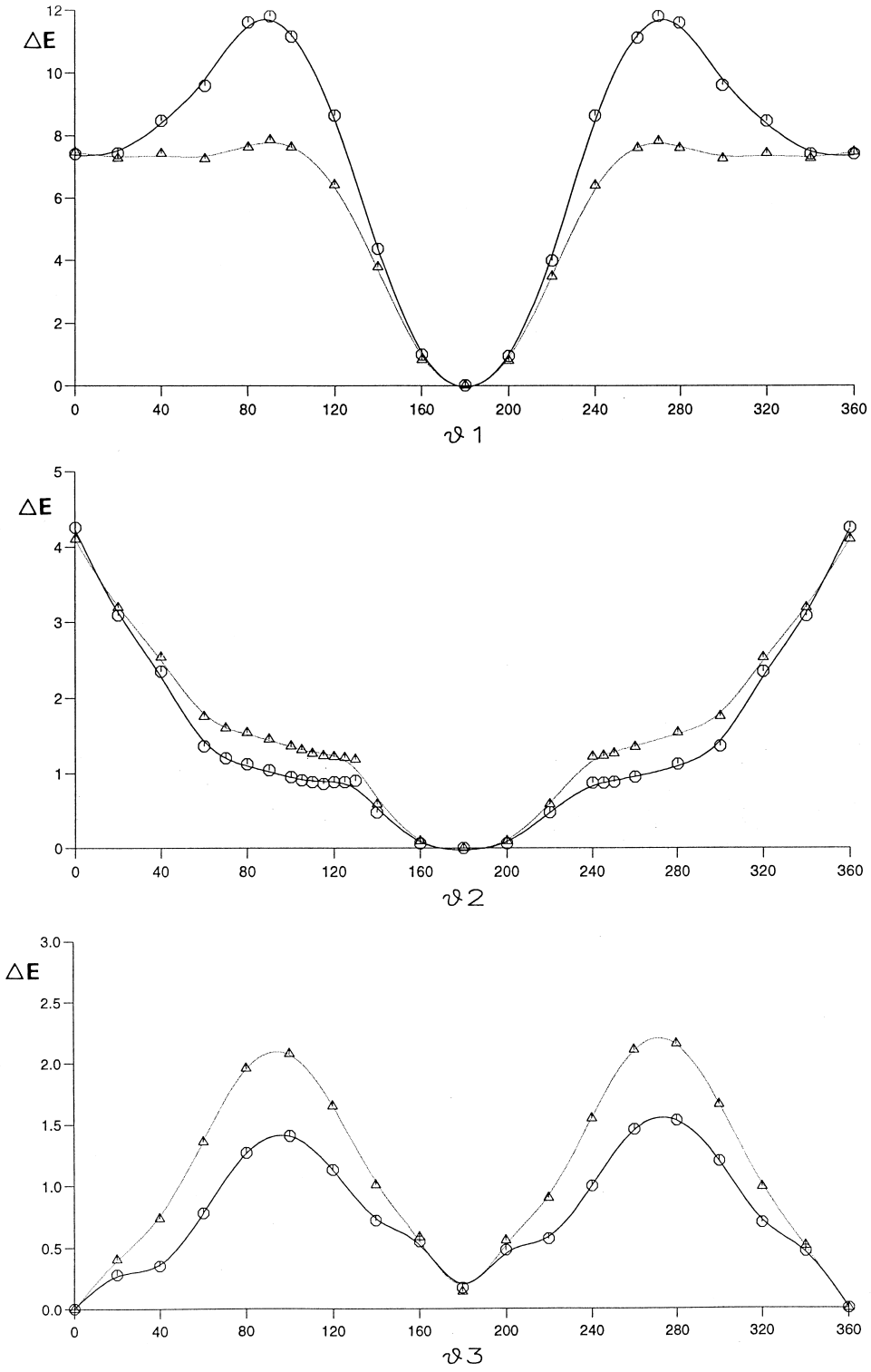


Figure 5. Energy profiles (kcal/mol) for the rotation around ϑ_1, ϑ_2 and ϑ_3 of the chalcone derivative **2** in vacuo (circles) and in water (triangles).

bonds to Thr113 in a way similar to that of the 4′-hydroxyl of the benzopyran-4-one derivative 1 (Fig. 1).

By comparing the binding modes of **2** (Fig. 6) and **1** (Fig. 1), we can conclude that these two classes of phenolic compounds, though having different chemical structures, bind the enzyme adopting very similar orientation and affording similar interactions with conserved protein residues. To conclude, when chalcone adopts the conformation revealed by conformational analysis, which differs from that observed crystallographically, but is energetically accessible, the structures of **1** and **2**

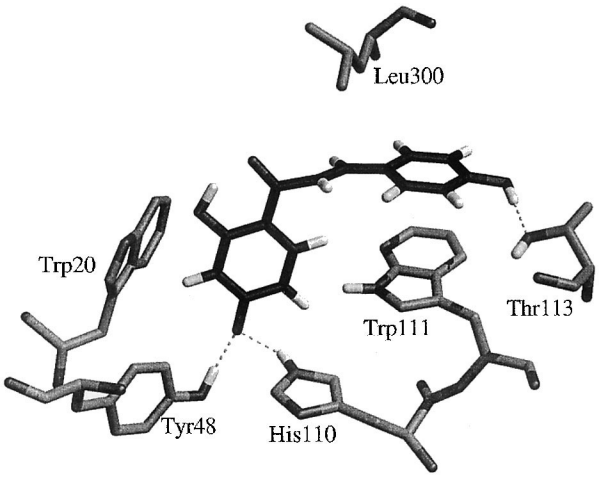


Figure 6. Enzyme residues interacting with the chalcone derivative **2** in the structure of the enzyme–inhibitor complex.

can be very well superimposed. Therefore, benzopyran-4-one derivatives and chalcones share similar structural bases for the inhibition of ALR2.

Conclusion

The determination of the acidity constants of a set of hydroxylated chalchonic compounds in relation to their ALR2 inhibitory activity supports the importance of the dissociated forms for biological activity; the comparative study of chalchones and benzopyran-4-one derivatives, conducted through the determination of acidity constants, crystal structures, conformational analysis and docking into the ALR2 structure, suggests how these inhibitors might bind to the enzyme. Overall, this study provides a rationale for the inhibition of ALR2 exerted by these two classes of compounds, and affords further insight into the development of phenolic compounds as inhibitors of this enzyme.

Experimental

Determination of the proton dissociation constants (pK_a)

The compounds being studied were dissolved in DMSO at a concentration of about 3.5 mM. Samples (5 μL) of this solution were added to 3 mL of buffer at a constant ionic strength[13] ($I = 0.1$ M) and with pH increasing each time in increments of 0.25 units; UV–vis spectra were recorded and the pH of the solution measured with a combined electrode (Orion SA 520) calibrated with buffers at pH 4.01, 7.00 and 10.01. The numerical values of the proton dissociation constants of the compounds under study were evaluated from the change in absorbance at the maximum λ of the undissociated (for **7**) and of the dissociated forms for the remaining compounds according to ref 14. pK'_a Values were then corrected for ionic strength as described therein.

X-ray diffraction analysis

The crystals of 7-methoxy-2-(4′-hydroxybenzyl)-4*H*-1-benzopyran-4-one (**8**) were grown from slow evaporation of an acetone solution. All X-ray measurements were carried out on a rotating-anode Siemens P4RA-M18X diffractometer (52 KV, 40 mA), under the conditions reported in Table 4. Unit cell parameters were derived from least-squares fit to the settings angles of 30 automatically centred reflections in the 5–16° ϑ range. Intensity data were corrected for Lorentz and polarization effects but not for absorption, in view of the low absorption coefficient and almost isotropic crystal dimensions.

The structure was solved by direct methods using the SHELX86[15] program and refined through full-matrix least-squares calculations based on F^2 by means of the SHELX93 program.[16] All non-H atoms were refined anisotropically, and hydrogen atoms, located in ΔF maps, isotropically, but with common temperature factors for methyl or non-methyl H atoms. Final reliability indices are reported in Table 4.

Lists of atomic coordinates, thermal parameters, bond distances and angles, torsion angles, selected least-squares planes and observed and calculated structure factors are available on request from the authors.

Conformational analysis and molecular mechanics simulations of the ALR2-chalcone complex

Conformational analysis was performed on 7-hydroxy-2-(4-hydroxybenzyl)-4*H*-1-benzopyran-4-one (**1**) dissociated at the 7-hydroxyl, and on 2′,4,4′-trihydroxychalcone (**2**) dissociated at the 4′-hydroxyl. The AMSOL

Table 4. Crystal data and structure refinement for 7-methoxy-2-(4′-hydroxybenzyl)-4*H*-1-benzopyran-4-one (**8**)

Empirical formula	$C_{17}H_{14}O_4$
Formula weight	282.28
Temperature	293 (2) K
Wavelength	0.71069 Å
Crystal system	Triclinic
Space group	$P\bar{1}$
Unit cell dimensions	$a = 11.015$ (2) Å $\alpha = 91.220$ (11)°
	$b = 11.3551$ (11) Å $\beta = 112.140$ (9)°
	$c = 13.3026$ (11) Å $\gamma = 111.720$ (8)°
Volume	1406.6 (3) Å^3
Z	4
Density (calculated)	1.333 Mg/m^3
Absorption coefficient	0.095 mm^{-1}
$F(000)$	592
Crystal size	0.30×0.25×0.25 mm
ϑ range for data collection	2.14 to 26.00°
Index ranges	$-13 \le h \le 13, -13 \le k \le 13, -1 \le l \le 16$
Reflections collected	6420
Independent reflections	4999 [$R_{int} = 0.028$]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4999/0/465
Goodness-of-fit on F^2	1.176
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0522$, $wR_2 = 0.1504$
R indices (all data)	$R_1 = 0.0665$, $wR_2 = 0.1560$
Extinction coefficient	0.019(2)
Largest diff. peak and hole	0.174 and −0.186 e. Å^{-3}

6.5.3[17] package and the PM3[18] parameterization were used. The rotational energy-profiles were obtained by rotating dihedral angles in steps of 20°, with complete energy minimization at each step. In some cases, the step was reduced to 10 or 5°.

Calculations in water were performed using the PM3-SM3 model,[19] with energy minimization performed at each step.

The structure of ALR2 used for docking and molecular mechanics calculations of the interaction with chalcone is that previously described for benzopyran-4-one derivatives.[3] Chalcone **2** was docked into the active site of the enzyme, in the conformation found to be particularly suited to binding ALR2, by positioning its dissociated 4′-hydroxyl close to Tyr48 and His110 and inserting the phenol ring into the additional hydrophobic pocket.[2,3] The AMBER 4.1[20] program with the Cornell et al.[21] force field was used for the energy-minimization of the complex. Van der Waals parameters of chalcone were assigned to be consistent with the Cornell force field, and atomic charges were calculated with an electrostatic potential fit to a 6-31G* ab initio wave function using Gaussian 94, followed by a standard RESP[22,23] fit.

Three thousand steps of conjugate gradinet minimization were performed, leaving all residues within 10 Å from the inhibitor free to move during minimization. A distant-dependent dielectric constant with a 4r dependance and a 10 Å non-bonded cutoff was adopted. Other details can be found in ref 3.

Calculations were performed on a Silicon Graphics O2 workstation.

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