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Synthesis and activity of a new series of chalcones as aldose reductase inhibitors

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Abstract – A new series of chalcone derivatives has been synthesized and tested in vitro in order to assess their ability to inhibit aldose reductase enzyme (ALR2) and their specificity towards the target enzyme with respect to other oxidoreductases, such as aldehyde reductase, sorbitol dehydrogenase, and glutathione reductase. All the compounds display affinity for ALR2. The X-ray crystal structure of 1-(2,4-dihydroxyphenyl)-3-(2-methoxyphenyl)propen-1-one was determined. © Elsevier, Paris

chalcones / aldose reductase inhibitors / sorbitol pathway / X-ray analysis

1. Introduction

The aldose reductase (alditol: $NADP^+$ oxidoreductase, EC 1.1.1.21, ALR2) is the first enzyme of the so-called 'polyol pathway'; in the presence of NADPH it converts glucose to sorbitol, which is further processed by sorbitol dehydrogenase (L-iditol: NAD^+ 5-oxidoreductase, EC 1.1.1.14, SD) to fructose [1]. Since ALR2 has low affinity for glucose, flux through this pathway is probably low in most tissues under normoglycemic conditions. On the contrary in diabetic conditions the blood glucose concentration increases sufficiently to provide a substrate for ALR2 in tissues such as nerve, lens, and retina, in which insulin is not necessary for glucose transport across the membrane. The increase glucose flux through the sorbitol pathway and/or the high intracellular accumulation of sorbitol are likely to be involved in the etiology of late-onset diabetic complicance such as neuropathy, nephropathy, retinopathy, and cataract [1, 2].

Several ALR2 inhibitors have been used to reduce or to delay the development of these diabetic complications, but many problems are associated with this therapy. The first concerns the specificity of the inhibition: ALR2 is a member of the aldo-keto reductase family, a group of enzymes that catalyze the NADPH-dependent reduction of a wide variety of carbonyl compounds, possessing a broad and overlapping substrate specificity. The possible consequences arising, in the course of chronic ALR2 inhibitors therapy, from the inhibition of closely related enzymes not involved in the polyol pathway are therefore of legetimate concern in the development of these drugs. The highest homology in structure and activity is seen with aldehyde reductase (alcohol: $NADP^+$ oxidoreductase, EC 1.1.1.2, ALR1) [3]. Even though the physiological role of this enzyme is not clear, it does not appear to participate significantly in polyol formation in vivo [4]. It is also important to determine the selectivity relative to other enzymes such as sorbitol dehydrogenase (SD) and/or glutathione reductase (GR) [2]: SD happens to be the second enzyme of the polyol pathway, and its contemporaneous inhibition would give rise to an intracellular accumulation of sorbitol, while the inhibition of GR would cause the accumulation of glutathione disulphide, which is able to modify ALR2, thus rendering it insensitive to the traditional inhibitors [5, 6].

Although numerous ALR2 inhibitors belong to different chemical classes, they have certain electronic and steric characteristics in common: in particular, the essen-

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tial requisites for this inhibitory effect would appear to be a planar structure with two hydrophobic moieties (aromatic groups) and the presence of an acidic proton, since both classes of the most important ALR2 inhibitors, cyclic imides and carboxylic acid, are likely to interact with the cationic site of the enzyme in their dissociated anionic forms [7].

Thus, the interesting discovery of the ALR2 inhibitory activity of phenolic compounds like chalcones, devoid of highly acidic carboxylic moiety [8] prompted us to study a series of isoliquiritigenin (2′,4,4′-trihydroxychalcone) analogous [9], taken as a lead compound, containing some modifications on aromatic rings (introduction of methoxy groups, halogen atoms or substitution with heteroaromatic rings) together with the corresponding dihydrochalcone derivatives (*table I*).

2. Chemistry

The compounds were synthesized according to the Claisen–Schmidt procedure which concerns the condensation of acetophenone with benzaldehyde derivatives. If hydroxy groups are present in aromatic rings they were protected, before the condensation, with 3,4-dihydro-α-pyran, followed by hydrolysis.

21–23 were obtained by reduction of the corresponding chalcones; **24** was synthesized by addition of thioglycolic acid to **14**.

Structural assigments for compounds are based on UV, mass spectral, ^{1}H-NMR, (*tables II–IV*) and X-ray diffraction data (*tables V–VIII*).

3. Biological assays

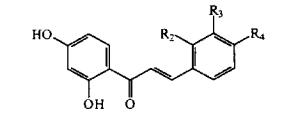
All the new compounds were studied in vitro for their ability to inhibit aldose reductase, sorbitol dehydrogenase, and two other enzymes, namely aldehyde reductase and glutathione reductase, which are not involved in the polyol pathway (*table IX*). Isoliquiritigenin, quercetin, Sorbinil, Tolrestat were used as reference compounds.

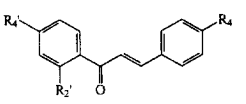
4. Results and discussion

4.1. X-ray analysis

With the aim to study the conformation of typical chalcones in order to examine the correpondence of these molecules with the pharmacophor requirements for ALR2 inhibitors [7], the model compound **8** was analysed by X-ray diffraction analysis.

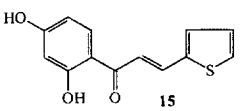
Table I.





1 R_2, R_3 = H, R_4 = OCH_3
2 R_2, R_3 = H, R_4 = SCH_3
3 R_2, R_3 = H, R_4 = Cl
4 R_2, R_4 = H, R_3 = OH
5 R_3 = H, R_2, R_4 = OCH_3
6 R_2 = H, R_3, R_4 = OCH_3
7 R_3, R_4 = H, R_2 = OCH_3
8 R_2, R_4 = H, R_3 = OCH_3
9 R_2 = H, R_3, R_4 = OH

10 R_4 = OCH_3, $R_{2'}$ = H, $R_{4'}$ = Br
11 R_4 = OCH_3, $R_{2'}$ = H, $R_{4'}$ = Cl
12 R_4 = Cl, $R_{2'}$, $R_{4'}$ = OCH_3
13 R_4, $R_{4'}$ = OCH_3, $R_{2'}$ = OH
14 R_4, $R_{2'}$, $R_{4'}$ = OCH_3



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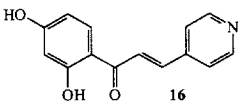

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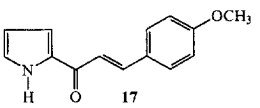

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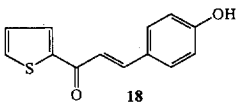

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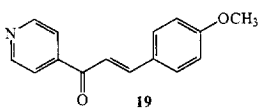

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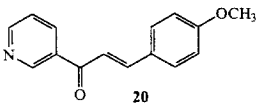

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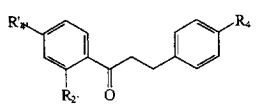
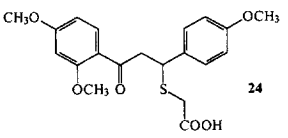




24

21 R_4, $R_{4'}$ = OCH_3, $R_{2'}$ = OH
22 R_4, $R_{2'}$, $R_{4'}$ = OCH_3
23 R_4, $R_{2'}$, $R_{4'}$ = OH

The asymmetric unit of **8** contains one independent molecule, presented in *figure 1* along with the atom numbering scheme. Although of limited accuracy, all bond distances and angles are typical [10]. The most interesting structural feature is a very flat molecular conformation: the maximum atomic deviations from the least-squares plane through all non-hydrogen atom range from –0.101(9) to +0.137(12) Å. The planes through the phenyl rings make dihedral angles of 3.1(5) and 2.9(5)°, respectively, with that through the atoms of the planar propene-1-one moiety: this planar structure seems to fit well in the hypothetical receptor. Also the methoxy group is nearly coplanar with respect to phenyl ring to which it is bonded, the C(13)–C(12)–O(4)–C(16) torsion angle being –1.9(17)°. The hydrogen bonding, which involves

Table II. Physical and chemical data of chalcone derivatives.

Compound	M.p. (°C)	λ_{max} (nm), logε	MS, *m/z*
1	165–168	364.0, 4.51	$270^{(100)}$, $269^{(61)}$, $253^{(12)}$, $163^{(26)}$, $137^{(30)}$, $121^{(89)}$, $108^{(26)}$
2	180–183	376.0, 4.53	$286^{(90)}$, $285^{(39)}$, $271^{(12)}$, $239^{(12)}$, $163^{(28)}$, $137^{(100)}$, $121^{(6)}$, $108^{(6)}$
3	179–182	320.0, 4.28	$274^{(100)}$, $273^{(80)}$, $239^{(12)}$, $163^{(99)}$, $137^{(79)}$, $108^{(23)}$
4	227–230	n.d. [a]	$256^{(100)}$, $255^{(73)}$, $239^{(23)}$, $227^{(6)}$, $163^{(95)}$, $137^{(80)}$, $120^{(12)}$, $119^{(7)}$, $108^{(7)}$
5	139–141	381.0, 4.26	$300^{(80)}$, $299^{(21)}$, $285^{(7)}$, $269^{(100)}$, $164^{(34)}$, $151^{(74)}$, $137^{(44)}$, $121^{(33)}$
6	209–211	376.0, 4.43	$300^{(100)}$, $299^{(40)}$, $285^{(12)}$, $269^{(11)}$, $164^{(58)}$, $151^{(95)}$, $137^{(27)}$
7	182–184	366.0, 4.40	$270^{(44)}$, $269^{(13)}$, $253^{(7)}$, $239^{(100)}$, $163^{(17)}$, $137^{(34)}$
8	130–132	356.0, 4.13	$270^{(100)}$, $269^{(62)}$, $253^{(4)}$, $239^{(18)}$, $163^{(90)}$, $137^{(43)}$, $108^{(18)}$
9	210–211	384.0, 4.21	$272^{(100)}$, $271^{(58)}$, $255^{(16)}$, $163^{(43)}$, $137^{(99)}$
10	138–140	348.1, 4.15	$317^{(73)}$, $316^{(43)}$, $237^{(100)}$, $161^{(64)}$, $156^{(19)}$, $133^{(38)}$
11	114–117	348.6, 4.10	$272^{(100)}$, $271^{(69)}$, $257^{(22)}$, $241^{(27)}$, $237^{(90)}$, $165^{(38)}$, $139^{(31)}$, $111^{(42)}$
12	105–108	312.2, 4.01	$302^{(59)}$, $301^{(20)}$, $287^{(23)}$, $274^{(42)}$, $177^{(33)}$, $165^{(100)}$
13	105–107	n.d. [a]	
14	90–92	n.d. [a]	$298^{(95)}$, $297^{(21)}$, $283^{(59)}$, $270^{(33)}$, $267^{(18)}$, $165^{(75)}$, $135^{(37)}$, $121^{(100)}$
15	143–146	369.0, 4.42	$246^{(100)}$, $245^{(47)}$, $229^{(20)}$, $217^{(14)}$, $137^{(46)}$, $110^{(68)}$
16	231–233	349.2, 3.44	$241^{(64)}$, $240^{(28)}$, $224^{(7)}$, $212^{(12)}$, $163^{(100)}$, $137^{(38)}$
17	126–128	356.1, 4.38	$227^{(100)}$, $226^{(48)}$, $212^{(33)}$, $198^{(12)}$, $119^{(12)}$
18	155–158	360.2, 4.33	$230^{(100)}$, $229^{(70)}$, $213^{(17)}$, $201^{(21)}$, $136^{(17)}$, $119^{(12)}$,$111^{(48)}$
19	100–103	355.2, 4.01	$239^{(100)}$, $238^{(22)}$, $224^{(44)}$, $208^{(48)}$, $161^{(100)}$, $133^{(69)}$
20	75–78	351.2, 4.22	$239^{(100)}$, $238^{(55)}$, $224^{(23)}$, $208^{(18)}$, $161^{(69)}$, $133^{(33)}$
21	53–54	n.d. [a]	n.d. [a]
22	74–76	302.4 (3.32)	$300^{(33)}$, $165^{(100)}$, $134^{(37)}$, $121^{(23)}$
23	191–193	n.d. [a]	n.d. [a]
24	47–50	n.d. [a]	n.d. [a]

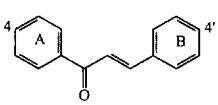
[a] Not determined.

both the OH functions and the ketonic O atom could play an important role in determining the planar conformation of the molecule. The O(3) atom acts as acceptor in a strong intramolecular hydrogen bond which involves the adjacent O(1)–H function. The same O(3) atom acts again as acceptor in a likewise strong intermolecular hydrogen bond interaction with the O(2)–H group. The O(1)···O(3) and O(2)···O(3) separations are 2.527(9) and 2.671(8) Å, respectively. The intermolecular hydrogen bond appears to be the major contributor to the molecular packing forces, although further contribution could arise from weak aromatic ring-stacking interactions between phenyl rings and propene-1-one moiety.

4.2. Enzyme section

The enzyme activity findings indicate that the great part of the compounds possess affinity for the ALR2 (*table IX*), although they are considerably less potent than tolrestat. IC_{50} was determined for **1**, **4**–**9**, **15**, **23** and **24** (IC_{50} values ranging from 6.43 to 27.60 μM) but could not be determined for the remaining chalcones owing to their poor solubility in the assay mixture.

The most active chalcones display two hydroxy groups in 2′–4′ of the A ring (**4**, **9**, **15**) according to the substitution pattern observed in the lead compound isoliquiritigenin (2′,4,4′-trihydroxychalcone) [9]:



The introduction of halogens on the rings causes a decrease of solubility in biological assay medium (**3**, **11**, **12**).

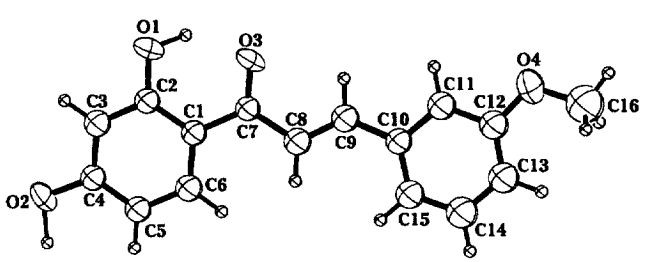


Figure 1. X-ray molecular structure of **8** with atom numbering scheme. Thermal parameters enclose 50% probability.

Table III. ^{1}H-NMR of **1–20**.

Compound	H2′	H3′	H4′	H5′	H6′	H2	H3	H4	H5	H6	Hα	Hβ	2′OH	4′OH	Other OH	OCH_3
1	–	6.39		6.52	8.29	7.97	7.13	–	7.13	7.97	7.87	7.94	13.63	10.76	–	3.92
2	–	6.39		6.52	8.29	7.94	7.43	–	7.43	7.94	7.86	8.03	13.56	10.81	–	2.63 SCH_3
3	–	6.40		6.53	8.30	8.04	7.63	–	7.63	8.04	7.87	8.09	13.43	10.84	–	–
4	–	6.39		6.52	8.28	7.31	–	6.98	7.35	7.43	7.81	7.98	13.46	10.81	9.70	–
5	–	6.38		6.51	8.22	–	6.75	–	6.73	8.04	7.89	8.17	13.71	10.74	–	3.95 4.01
6	–	6.39		6.49	8.31	7.65	–	–	7.12	7.49	7.86	7.94	13.67	10.77	–	3.94 3.97
7	–	6.40		6.53							8.02	8.22	13.53	10.81	–	4.01
8	–	6.40		6.53	8.34	7.60	–	7.13	7.47	7.54	7.87	8.08	13.49	10.84	–	3.94
9	–	6.37		6.50	8.23	7.36	–	–	6.91	7.31	7.76	7.76	13.67	10.73	9.18 9.83	–
10	8.09	7.79		7.79	8.09	7.87	7.04	–	7.04	7.87	7.76	7.76	–	–	–	3.85
11	8.17	7.64		7.64	8.17	7.87	7.04	–	7.04	7.87	7.57	7.57	–	–	–	3.85
12	–	6.71		6.66	7.64	7.76	7.50	–	7.50	7.76	7.55	7.55	–	–	–	3.87 3.92
13	–	6.91		6.84	7.66	7.14	6.67	–	6.67	7.14	7.81	7.92	13.58	–	–	3.85 3.87
14	–	6.71		6.66	7.61	7.69	7.01	–	7.01	7.69	7.40	7.54	–	–	–	3.83 3.87 3.92
15	–	6.39	–	6.52	8.18	–	7.80	7.30	7.91	–	7.72	8.06	13.42	10.81	–	
16	–	6.33	–	6.46	8.18	8.68	7.85	–	7.85	8.68	7.73	8.19	13.14	10.85	–	–
17	–	7.16	7.32	6.28	–	7.80	7.02	–	7.02	7.80	7.55	7.65	–	–	NH 11.90	–
18	–	8.26	–	8.02	7.31	7.74	7.51	–	7.51	7.74	7.67	7.67	–	10.08	–	
19	8.83	7.98	–	7.98	8.83	7.89	7.05	–	7.05	7.89	7.77	7.77	–	–	–	3.85
20	9.26	–	8.43	7.60	8.83	7.89	7.04	–	7.04	7.89	7.81	7.81	–	–	–	3.85

Table IV. ^{1}H-NMR of **21–24**.

Compound	H3′	H5′	H6′	H2	H3	H5	H6	CH_2	CH_2	2′OH	4′OH	Other OH	OCH_3
21	6.45	6.53	7.87	7.24	6.85	6.85	7.24	3.33	3.01	12.80	–	–	3.78 3.90
22	6.60	3.66	7.63	7.14	6.83	6.83	7.14	3.15	2.83	–	–	–	3.72 3.85 3.89
23	6.37	6.25	7.80	7.05	6.67	6.67	7.05	3.20	2.84	12.62	10.54	9.18	–
24	6.65	6.57	7.51	7.23	6.86	6.86	7.23	3.00	3.05	–	–	–	3.73 3.84 3.93

The saturation of the α–β double bond decreased the ALR2 inhibitor activity in methoxylated compounds (compare **13** and **14**, with **21** and **22**), while in the case of **23,** a trihydroxylated compound, the activity is the same as its unsaturated corresponding compound isoliquiritigenin.

Substitution of the B ring with thiophen (**15**) does not modify the activity (IC_{50} = 9.43 μM), whereas the same group substituted with the A ring (**18**) decreases the activity (32% = 68 μM).

The substitution of the aromatic rings with other heterocycles (pyrrol and pyridine) (**17, 19, 20**) led to derivatives chracterized by low activity and low solubility.

The introduction of thioglycolic group (compound **24**) in the chalconic structure **14** gave an increase in inhibitor

Table V. Crystal data and structure refinement for **8**.

Identification code	Compound **8**
Empirical formula	$C_{16}H_{14}O_4$
Formula weight	270.27
Temperature	293(2) K
Wavelength	0.71069 Å
Crystal system	orthorhombic
Space group	$Pca2_1$
Unit cell dimensions	$a = 16.428(3)$ Å $b = 4.009(1)$ Å $c = 20.272(3)$ Å
Volume	1335(1) Å^3
Z	4
Density (calculated)	1.345 Mg/m^3
Absorption coefficient	0.097 mm^{-1}
$F(000)$	568
Crystal size	0.15 x 0.10 x 0.10 mm
θ range for data collection	2.01° to 21.99°
Index ranges	$-1 \le h \le 16$, $-1 \le k \le 4$, $-1 \le l \le 21$
Reflections collected	1515
Independent reflections	722 [$R_{int} = 0.031$]
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	722 / 0 / 105
Goodness-of-fit on F^2	1.054
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0635$, $wR2 = 0.1527$
R indices (all data)	$R1 = 0.0723$, $wR2 = 0.1593$
Absolute structure parameter	0(5)
Largest diff. peak and hole	0.203 and –0.214 e Å^{-3}

Table VI. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for compound **8**. U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	y	z	U_{eq}
O(1)	56(3)	–3039(17)	3237(5)	63(2)*
O(2)	2874(3)	–2600(17)	3477(5)	60(2)*
O(3)	–826(3)	–48(18)	4069(5)	58(2)*
O(4)	–3385(4)	7242(23)	6425(6)	76(2)*
C(1)	592(5)	–170(23)	4180	39(2)
C(2)	690(5)	–1975(22)	3598(6)	40(2)
C(3)	1457(5)	–2768(24)	3379(6)	45(2)
C(4)	2139(5)	–1736(23)	3731(6)	41(2)
C(5)	2059(5)	43(22)	4296(5)	42(2)
C(6)	1292(5)	834(22)	4535(6)	45(2)
C(7)	–233(5)	748(21)	4411(5)	38(2)
C(8)	–357(5)	2606(21)	5031(6)	47(2)
C(9)	–1100(5)	3521(24)	5206(6)	48(2)
C(10)	–1381(5)	5213(25)	5793(6)	43(2)
C(11)	–2235(5)	5589(24)	5863(6)	50(2)
C(12)	–2550(5)	7152(26)	6412(6)	53(2)
C(13)	–2037(6)	8327(27)	6884(6)	59(3)
C(14)	–1206(7)	7954(26)	6819(7)	64(3)
C(15)	–881(6)	6436(25)	6275(6)	51(2)
C(16)	–3748(8)	8627(35)	7002(7)	92(4)

* Only these atoms were treated anisotropically.

activity ($IC_{50} = 6.43$); thus, the presence of an acid function in addition of dihydrochalcone structure, appears an essential requirement for the activity.

All the compounds showed good selectivity towards SD ($IC_{50} > 150$ μM, Tolrestat 120 μM at 16.4%, quercetin and Sorbinil $IC_{50} > 150$ μM) and GR ($IC_{50} > 130$ μM, Sorbinil, Tolrestat and quercetin $IC_{50} > 150$ μM); on the contrary they show a marked activity on ALR1 (*table IX*).

Alltogether, these data show that for an efficient inhibition of ALR2 the presence of hydroxy groups in the A ring is important; in the case of their absence, the presence of a carboxylic moiety in the molecule suggests the importance of a ionized group for the interaction with ALR2.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Buchi 510 apparatus and are uncorrected. The UV spectra were recorded on a Perkin Elmer Lambda 15 spectrophotometer using 1 cm quartz cells in a 10^{-5} M ethanol solution. The wavelength absorption maxima are reported in nanometer. The ^{1}H-NMR spectra were recorded in DMSO-d_6 or in CD_3OD solution with a Bruker AMX-400 WB. Chemical shifts are reported in p.p.m. from tetramethylsilane used as internal standard, and are given in δ units. Mass spectra were performed with a Finningan MAT SSQ 710 instrument.

Microanalysis were carried out in the Microanalysis Laboratory of the Dipartimento di Scienze Farmaceutiche, Modena University (Italy) and were within ±0.4% of the theoretical values.

The compounds were separated by flash-chromatography with silica gel 60 (particle size 0.040–0.063 mm) (Merck) and the column was connected to an LKB Multirac 2111 fraction collector. The fractions were monitored using TLC plates.

5.1.1. General procedure for the preparation of chalcone ***1–20***

Synthesis of ***4***: 3-Hydroxybenzaldehyde (6.60 mmol) and pyridinium-*p*-toluenesulfonate (0.16 mmol) were suspended in methylene chloride (30 mL), and 3,4-dihydro-α-pyran (19.73 mmol) in methylene chloride (10 mL) was added dropwise and stirred at room temperature for 24 h. The reaction mixture was washed with water, and then the organic phase was dried (Na_2SO_4), concentrated in vacuo and purified by flash-chromatography (cyclohexane/ethyl acetate 9:2) to obtain the corresponding 3-(tetrahydropyran-2-yloxy) benzaldehyde (yield 77%).

2,4-Dihydroxyacetophenone (6.60 mmol) and pyridinium-*p*-toluenesulfonate (0.16 mmol) were suspended in methylene chloride (30 mL), and 3,4-dihydro-α-pyran (19.73 mmol) in methylene chloride (30 mL) was added dropwise and stirred at room tempera-

Table VII. Bond lengths [Å] and angles [deg] for compound **8**.

C(1)–C(2)	1.394(12)	C(1)–C(6)	1.416(11)
C(1)–C(7)	1.480(11)	C(2)–O(1)	1.342(10)
C(2)–C(3)	1.373(12)	C(3)–C(4)	1.392(12)
C(4)–C(5)	1.357(12)	C(4)–O(2)	1.357(10)
C(5)–C(6)	1.387(11)	C(7)–O(3)	1.237(10)
C(7)–C(8)	1.475(11)	C(8)–C(9)	1.322(12)
C(9)–C(10)	1.445(13)	C(10)–C(15)	1.368(13)
C(10)–C(11)	1.419(12)	C(11)–C(12)	1.378(13)
C(12)–C(13)	1.359(13)	C(12)–O(4)	1.373(10)
C(13)–C(14)	1.378(14)	C(14)–C(15)	1.368(13)
O(4)–C(16)	1.424(14)		
C(2)–C(1)–C(6)	119.0(7)	C(2)–C(1)–C(7)	120.2(7)
C(6)–C(1)–C(7)	120.8(7)	O(1)–C(2)–C(3)	117.5(8)
O(1)–C(2)–C(1)	122.5(7)	C(3)–C(2)–C(1)	120.0(8)
C(2)–C(3)–C(4)	120.4(8)	C(5)–C(4)–O(2)	122.8(8)
C(5)–C(4)–C(3)	120.7(8)	O(2)–C(4)–C(3)	116.5(8)
C(4)–C(5)–C(6)	120.2(8)	C(5)–C(6)–C(1)	119.7(8)
O(3)–C(7)–C(8)	119.9(7)	O(3)–C(7)–C(1)	118.6(7)
C(8)–C(7)–C(1)	121.4(7)	C(9)–C(8)–C(7)	119.7(9)
C(8)–C(9)–C(10)	130.2(9)	C(15)–C(10)–C(11)	118.9(9)
C(15)–C(10)–C(9)	124.4(8)	C(11)–C(10)–C(9)	116.7(8)
C(12)–C(11)–C(10)	120.0(9)	C(13)–C(12)–O(4)	126.7(9)
C(13)–C(12)–C(11)	119.5(9)	O(4)–C(12)–C(11)	113.7(8)
C(12)–C(13)–C(14)	120.6(10)	C(15)–C(14)–C(13)	120.8(10)
C(14)–C(15)–C(10)	120.1(9)	C(12)–O(4)–C(16)	116.4(9)

Table VIII. Hydrogen bond interactions in compound **8**.

Atoms			1---3	1–2	2---3	/1–2---3
1	2	3				
O(1)–H(O1)---O(3)			2.527(9)	1.03	1.86	126
O(2)–H(O2)---O(3[a])			2.671(8)	1.22	1.48	165

[a] Symmetry transformation $0.5 + x$, $-y$, z of the reference coordinates.

ture for 12 h. The reaction mixture was washed with water, dried (Na_2SO_4), concentrated in vacuo and then purified by flash chromatography (cyclohexane/ethyl acetate 9:2) to obtain 2-hydroxy-4-(tetrahydropyran-2-yloxy) acetophenone (yield 90%, m.p. 67–69 °C).

2-Hydroxy-4-(tetrahydropyran-2-yloxy) acetophenone (4.24 mmol), 3-(tetrahydropyran-2-yloxy) benzaldehyde (2 equivalent) were dissolved in methanol (10 mL) and barium hydroxide octahydrate (6.3 mmol) in methanol (50 mL) were slowly added and stirred for 24 h at 40 °C. The reaction mixture was concentrated in vacuo. After, water (100 mL) was added to the mixture, then neutralized with 1 M HCl and extracted with ethyl acetate. The organic layer was separated, washed, dried (Na_2SO_4) and evaporated in vacuo. This residue yielded crude 2′-hydroxy-3,4′-di(tetrahydropyran-2-yloxy) chalcone.

The compound was suspended in methanol (30 mL) and *p*-toluene sulfonic acid (0.1 mmol) was added. The reaction mixture was stirred for 1 h at room temperature. After water (30 mL) had been added to the mixture it was neutralized with Na_2CO_3 and extracted with ethyl acetate. The organic layer was separated, washed with water, dried (Na_2SO_4) and evaporated in vacuo. The residue was separated by flash-chromatography (toluene/acetone 4:1) to give **4**.

The same procedure was applied to obtain **9** starting from 3,4-dihydroxybenzaldehyde.

The other dihydroxychalcones **1–3** and **5–8** were obtained by condensation of 2-hydroxy-4-(tetrahydropyran-2-yloxy) acetophenone with the respective benzaldehyde, while **18** was synthesized from respective acetophenone with 4-(tetrahydropyran-2-yloxy) benzaldehyde.

Table IX. Enzyme inhibition data [a].

Compound	ALR2	ALR1
1	27.60	4.41
2	39% (13.27)	[b]
3	35% (30.03)	[b]
4	10.44	0.77
5	12.19	2.69
6	11.59	5.57
7	12.44	1.98
8	13.75	2.40
9	7.33	0.70
10	[c]	[c]
11	[c]	[c]
12	[c]	[c]
13	40% (78.54)	[b]
14	10% (18.51)	[b]
15	9.43	[b]
16	[d]	[d]
17	0% (44.00)	[b]
18	32% (68.50)	[b]
19	0% (40.12)	[b]
20	0% (44.26)	[b]
21	0% (42.00)	[b]
22	0% (20.00)	[b]
23	12.70	1.00
24	6.43	1.41
Isoliquiritigenin	7.60 [9]	0.93
Quercetin	39.9	0.3
Sorbinil	3.04	1.74
Tolrestat	0.096	1.21

[a] IC_{50} expressed in μM or percent inhibition (at a given concentration).
[b] Not tested.
[c] The compounds are insoluble in the biological medium.
[d] The compound changes in biological assay conditions.

Compounds **10–14**, **17**, **19**, **20** were prepared following the same procedure starting from the corresponding substituted acetophenone and benzaldehyde without protection.

All the compounds were crystallized from methanol/water.

*5.1.2. Synthesis of **21–23** dehydrochalcones*

The compounds **21–23** were obtained by reduction of 3.4 mM of the appropriate chalcones (**13**, **14**; for **23** see [9]) with 10% Pd/C in cyclohexene at reflux for 1 h. The suspension was filtered and the residue was washed with ethyl acetate. The mixture was separated by flash-chromatography with cyclohexane/ethyl acetate (9:2) and the product was crystallized from ethanol.

*5.1.3. Synthesis of [3-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)-3-oxopropyl sulphanyl] acetic acid **24***

Thioglicolic acid (5.4 mmol) was added to **14** (3.3 mmol) in toluene (30 mL) and the mixture was warmed at reflux for 6 h. The crude was separated by flash-chromatography on silica gel with cyclohexane/ethyl acetate/acetic acid (9:3:0.1) as mobile phase.

*5.1.4. X-ray diffraction analysis of 1-(2,4-Dihydroxy-phenyl)-3-(3-methoxy-phenyl)-propene-1-one **8***

Molecular formula $C_{16}H_{14}O_4$; molecular weight 270.27; crystallized from acetone/H_2O in the orthorhombic space group $Pca2_1$ (No. 29). The unit cell parameters were $a = 16.428(3)$, $b = 4.009(1)$, $c = 20.272(3)$ Å, $V = 1335(1)$ Å^3, $Z = 4$, $D_{calc} = 1.345$ Mg m^{-3}, and $F(000) = 568$. Great difficults were encountered in obtaining crystals suitable for X-ray analysis. The compound was recrystallized from many solvents, but always very small crystals, moreover largely affected by extensive gemination, were obtained. Only one crystal proved to be of sufficient quality for intensity data collection. Its approximate dimensions were 0.15 × 0.10 × 0.10. All X-ray measurements were carried out on a Siemens P4RA-M18X diffractometer (52 KV, 130 mA) at room temperature by using Mo $K\alpha$ radiation ($\lambda = 0.71609$ Å). Unit cell parameters were derived from least-squares fit to the setting angles of 26 automatically centred reflections in the 5–13° θ range. A total of 1515 reflections were collected using the ω – 2θ scan mode in the 2–22° θ range; 961 reflections had $I \geq 2\sigma(I)$ and 722 were unique ($R_{int} = 0.031$). Absorption correction was not applied to intensities ($\mu = 0.097$ mm^{-1}). The structure was solved by direct methods using the SHELX86 [11] program and refined through full-matrix least-squares calculations by means of the SHELX93 program [12]. Because of an extremely low reflection/parameter ratio, only the O atoms were refined anisotropically, whereas the C atoms were treated isotropically. C-bonded H atoms were placed in calculated positions and O-bonded hydrogens treated as fixed contributors in observed positions. Least-squares refinement of 105 parameters led to final R and $wR2$ values of 0.0635 and 0.1527, respectively.

Lists of anisotropic displacement parameters, hydrogen coordinates and isotropic displacement parameters, torsion angles, selected least squares planes, and observed and calculated structure factors are available on request from the authors.

5.2. Enzyme section

Glutathione reductase (GR) type IV from yeast (100–300 U/mg), pyridine coenzymes, D,L-glyceraldehyde, glutathione disulfide, dithithreitol (DTT), sorbitol D-glucuronate, and sodium valproate were purchased from Sigma. DEAE-cellulose (DE-52) was obtained from Whatman. Sephadex G75 resin was from Pharmacia Biotech. Orange Matrex A, centricon-10 microconcentrators, and YM10 ultrafiltration membranes were from Amicon. Sorbinil was a gift from Pfizer. Quercetin was purchased from Fluka. Sorbitol dehydrogenase (SD) from sheep liver (10 U/mg of protein) was from Boehringer.

Protein concentration was determined according to the method of Bradford [13], using bovine serum albumin method as standard. In order to minimize cross-contamination between ALR2 and ALR1 in the enzyme preparations, bovine lens, which contains a significantly high proportion of ALR2 over ALR1, and bovine kidney, in which ALR1 is the predominant enzyme [14], were selected for isolation of ALR2 and ALR1, respectively. Calf lenses and kidneys for the purification of ALR2 and ALR1 were obtained locally from freshly slaughtered animals.

5.2.1. Preparation of aldose reductase (ALR2)

In order to purify the native form of bovine lens aldose reductase, the capsule was incised and the frozen lens was

suspended in sodium potassium phosphate buffer pH 7 (standard buffer), containing 5 mM DTT (1 g of tissue/3.5 mL) and stirred in an ice-cold bath for 1 h. The suspension was then centrifuged at 22000 *g* at 4 °C for 40 min and the supernatant subjected to ion exchange chromatography on DE52, as previously described [15]. Partially purified enzyme preparations with a specific activity of 6.5 mU/mg were routinely used to test enzyme inhibition; no appreciable ALR1 contamination was detected by sodium valproate assay [14]. At this stage of purification ALR2 could be stored at –20 °C without loss of activity for at least 1 month.

5.2.2. Preparation of aldehyde reductase (ALR1)

Partially purified ALR1 was obtained following a previously reported method [14]. Bovine kidneys were homogenized in 3 volumes of 0.25 M sucrose, 2.0 mM EDTA dipotassium salt, and 2.5 mM β-mercaptoethanol in 10 mM sodium phosphate buffer, pH 7.2 (S-buffer). The homogenate was centrifuged (16000 *g* for 20 min at 4 °C) and the supernatant subjected to ammonium sulfate fractional precipitation. The pellet obtained between 45% and 75% of salt saturation, containing ALR1 activity, was redissolved in S-buffer containing 2.0 mM EDTA (dipotassium salt) and 2.0 mM β-mercaptoethanol at a protein concentration of approximately 20 mg/mL. DEAE-52 resin was added to the solution and then removed by centrifugation; the supernatant was then stored at –20 °C. The enzyme preparation at this step of purification displayed a specific activity of 11.3 mU/mg and appeared devoid of any ALR2 activity, being ineffective in reducing glucose. The enzyme can be stored at –20 °C without loss of activity for at least 1 month.

5.2.3. Enzyme activity measurements

Enzyme activity for all tested enzymes was measured by monitoring the change in absorbance at 340 nm which accompanies the oxidation of NADPH or the reduction of NAD^+ catalyzed by ALR2, ALR1, GR, and SD, respectively. One unit of enzyme activity for all the tested enzymes is the amount of the enzyme which catalyzes the oxidation or the reduction of 1 μmol of the appropriate pyridine cofactor/min in the specified assay conditions. The assay for ALR2 activity was performed at 37 °C as previously described [16] using 4.7 mM D,L-glyceraldehyde as substrate in 0.25 M sodium phosphate buffer, pH 6.8, containing 0.38 M ammonium sulfate and 0.11 mM NADPH. ALR1 activity and 0.12 mM NADPH in 0.1 M sodium phosphate buffer, pH 7.2 [17]. SD activity was determined at 25 °C in 50 mM sodium phosphate buffer, pH 7.0 using 10 mM sorbitol as substrate and 0.47 mM NAD^+ [18]. GR activity was assayed at 30 °C in 0.125 M sodium phosphate buffer, pH 7.4, supplemented by 6.3 mM potassium EDTA, in the presence of 0.3 mM glutathione disulfide and 0.36 mM NADPH [19].

5.2.4. Enzyme inhibition

The compounds under study were solved in DMSO, which was maintained at constant concentration (1% final concentration) in the assays and in the reference reactions. The inhibitory activity of the compounds was determined by including the inhibitor solution in DMSO. A reference blank containing all the above reagents except the substrate was used to correct for the oxidation of NADPH not associated with the catalytic activity. The enzyme concentrations in the inhibition studies were as follows: 3.5 mU/mL for ALR2 and ALR1, 3.75 mM/mL for SD, and 4.5 mU/mL for GR. IC_{50} values (the concentration of the inhibitor required to produce 50% inhibition of the enzyme-catalyzed reaction) were determined from least-squares analyses of the linear portion of the log dose–inhibition curves. Each curves was generated using at least three concentration of inhibitor causing an inhibition between 20% and 80% with two replicates at each concentration.

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