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6,7-Dihydroxy-4-phenylcoumarin as inhibitor of aldose reductase 2

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ABSTRACT

We report the structure–activity relationship of a series of coumarins as aldose reductase 2 (ALR2) inhibitors and their suppressive effect on the accumulation of galactitol in the rat lens. We evaluated their ALR2 selectivity profile against sorbitol dehydrogenase and aldehyde reductase (ALR1). Our study revealed that substitutions in the C7 OH group enhanced the potency toward ALR2, while the C6 OH group interferes with ALR1 inhibition activity. Having the phenyl moiety at C4 leads to improved potency and improved selectivity. A molecular docking study suggested that 6,7-dihydroxy-4-phenylcoumarin (**15**) binds to ALR2 in a different manner from epalrestat. Furthermore, compound **15** clearly suppressed galactitol accumulation in a dose-dependent manner. These results provide an insight into the structural requirements of coumarins for developing a new-type of selective ALR2 inhibitor.

Prolonged hyperglycemia is a primary causal factor of several diabetic complications. Many studies have revealed a correlation between glucose metabolism via polyol pathway and long-term complications.[1,2] Aldose reductase (alditol: $NADP^+$ 1-oxidoreductase, EC 1.1.1.21, ALR2) and sorbitol dehydrogenase (L-iditol: NAD^+ 5-oxidoreductase, EC 1.1.1.14, SDH) play key roles in this pathway. Among them, ALR2 belongs to the aldo-keto reductase superfamily and catalyzes the reduction of glucose to sorbitol.[3,4] ALR2 has a low affinity for glucose and the polyol pathway plays a minor role in glucose metabolism because it competes with the glucose hexokinase of the glycolytic pathway. In the hyperglycemic condition, however, sufficient glucose can enter the tissues, and the pathway operates to produce the sorbitol and it is formed more rapidly than it is converted to fructose by SDH. Thus, sorbitol accumulates in the cells because of its poor penetration across the membranes. These abnormal metabolic results have been reported to be factors responsible for diabetic complications such as cataracts,[5] retinopathy,[6] neuropathy,[7] and nephropathy.[8] Therefore, ALR2 inhibitors have considerable potential for the treatment of these diseases, without increasing risk of hypoglycemia. Although many ALR2 inhibitors have been reported in the literature[9–12] and ranirestat (AS-3201) is in late-stage clinical development,[13,14] only epalrestat is successfully marketed for treatment of diabetic neuropathy in Japan. These failures are generally due to either a lack of efficacy or the emergence of unacceptable side effects. In some cases, the side effects are caused from a lack of selectivity relative to aldehyde reductase (EC 1.1.1.2, ALR1). ALR1 is also a member of the aldo-keto reductase superfamily and it shares a high degree of structural homology with ALR2, with 65% similarity in their amino acid sequences.[15] ALR1 plays important roles in not only detoxification such as of 3-deoxyglucosone, which is intermediate in the formation of toxic advanced glycation end products, but it also regulates an inflammatory response through the metabolism of prostaglandins.[16,17] It is therefore necessary to elucidate the structural requirements for the design of selective ALR2 inhibitors without affecting the detoxification activity of ALR1. In the course of a search for selective ALR2 inhibitors we considered the coumarins since their inhibitory effects on ALR2 have been reported and many kinds of plants produce them (Fig. 1).[18] However, previous attempts to explain the relationship of their structure and their inhibition of ALR2 have been inconclusive. Our study involved a molecular docking study of coumarins and study of the suppressive effects on the accumulation of galactitol in rat lens.

In the first experiment, we examined the inhibition activities of mono-, and dihydroxylated coumarins (**1**–**9**).[19,20] Table 1 shows the 50% inhibitory concentrations (IC_{50}) of these compounds against ALR2, SDH, and ALR1, and the resulting selectivity index (SDH/ALR2 and ALR1/ALR2) for each compound. The basic structure, coumarin (**1**) itself exhibited less than 50% inhibition of ALR2, even at concentrations as high as 400 μM, whereas 6,7-dihydroxycoumarin (**7**) was a good inhibitor with an IC_{50} value of 36.5 μM. In contrast,

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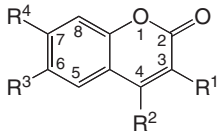

	R^1	R^2	R^3	R^4
Coumarin **(1)**	H	H	H	H
3-Hydroxycoumarin **(2)**	OH	H	H	H
4-Hydroxycoumarin **(3)**	H	OH	H	H
6-Hydroxycoumarin **(4)**	H	H	OH	H
7-Hydroxycoumarin **(5)**	H	H	H	OH
7-Methoxycoumarin **(6)**	H	H	H	OCH_3
6,7-Dihydroxycoumarin **(7)**	H	H	OH	OH
7-Hydroxy-6-methoxycoumarin **(8)**	H	H	OCH_3	OH
6-Hydroxy-7-methoxycoumarin **(9)**	H	H	OH	OCH_3
7-Hydroxy-4-methylcoumarin **(10)**	H	CH_3	H	OH
7-Hydroxylcoumarinyl-4-acetic acid **(11)**	H	CH_2COOH	H	OH
7-Hydroxy-4-phenylcoumarin **(12)**	H	C_6H_5	H	OH
6,7-Dihydroxy-4-methylcoumarin **(13)**	H	CH_3	OH	OH
6,7-Dihydroxycoumarinyl-4-acetic acid **(14)**	H	CH_2COOH	OH	OH
6,7-Dihydroxy-4-phenylcoumarin **(15)**	H	C_6H_5	OH	OH

Figure 1. Structure of coumarins.

Table 1
Concentration of coumarins giving 50% inhibition of ALR2, SDH, and ALR1 activities and their selectivity index against ALR2

	IC_{50} (μM)			Selectivity index	
	ALR2	SDH	ALR1	(SDH/ALR2)	(ALR1/ALR2)
1	>400	>400	>500	—	—
2	172	106	62.0	0.62	0.35
3	40.5	>400	>400	>9.88	>9.88
4	156	>400	171	>2.56	1.10
5	85.7	120	22.9	1.40	0.27
6	131	124	67.5	0.95	0.52
7	36.5	82.9	67.2	2.27	1.84
8	46.0	137	74.4	2.98	1.62
9	101	>250	56.2	>2.48	0.56
10	89.9	153	24.5	1.70	0.27
11	142	163	20.8	1.15	0.15
12	36.7	70.7	2.4	1.93	0.07
13	38.3	212	64.4	5.54	1.68
14	53.4	335	63.3	6.27	1.19
15	9.6	288	66.3	30.0	6.90
Epalrestat	1.4	67.7	12.5	48.4	8.93

6-hydroxycoumarin (**4**: IC_{50} = 156 μM) and 7-hydroxycoumarin (**5**: IC_{50} = 85.7 μM) were found to be much weaker than 6,7-dihydroxycoumarin (**7**). Especially, compound **4** which was four times weaker as an inhibitor than **7**. From a comparison of the potency of 7-hydroxycoumarin (**5**), 7-methoxycoumarin (**6**), 6,7-dihydroxycoumarin (**7**), 7-hydroxy-6-methoxycoumarin (**8**) and 6-hydroxy-7-methoxycoumarin (**9**), the OH substituent at the C7-position enhanced the potency against ALR2. 6,7-dihydroxycoumarin (**7**) also showed moderate inhibition towards ALR1 with an IC_{50} value of 67.2 μM. Interestingly, 6-hydroxycoumarin (**4**) gave lower inhibition (171 μM), while 7-hydroxycoumarin (**5**) had a threefold greater IC_{50} (22.9 μM) than 6,7-dihydroxycoumarin (**7**). This behavior is similar to that observed for ALR2 inhibition. Thus, the substituent at the C7-position is a key factor in obtaining improved potency of ALR2 inhibitors. Furthermore, the comparison of selectivity index of 3-hydroxycoumarin (**2**), 4-hydroxycoumarin (**3**), 6-hydroxycoumarin (**4**), and 7-hydroxycoumarin (**4**) led us to assume that the C4-position is another essential feature for recognition of ALR2 and could affect the selectivity.

Thus, we therefore focused on the C4-position and whether the introduction of different substitutions at this position could influence the inhibition activities of 7-hydroxycoumarin (**5**) and 6,7-dihydroxycoumarin (**7**) (Table 1). Of these compounds, the introduction of a methyl group such as in 7-hydroxy-4-methylcoumarin (**10**) and 6,7-dihydroxy-4-methylcoumarin (**13**) did not influence their ALR2 inhibition activities. In contrast, remarkable reductions of ALR2 inhibition were observed in 7-hydroxylcoumarinyl-4-acetic acid (**11**) and 6,7-dihydroxycoumarinyl-4-acetic acid (**14**) (142 and 53.8 μM, respectively), while introduction of a phenyl group such as 7-hydroxy-4-phenylcoumarin (**12**) and 6,7-dihydroxy-4-phenylcoumarin (**15**) had a pronounced effect on increasing potency of inhibition. Notably, 6,7-dihydroxy-4-phenylcoumarin (**15**) was around four times more effective than compound **7** with an IC_{50} of 9.6 μM. In addition, comparison of 6,7-dihydroxy compounds (**13**–**15**) and the 6-deoxy compounds (**10**–**12**) revealed that the C6 OH group interferes with ALR1 inhibition activities but improves the recognition toward ALR2. These results would indicate that a substituent at the C6-position is a dominant factor for the selective inhibition of ALR2. 6,7-Dihydroxy-4-phenylcoumarin (**15**) examines further the selectivity possibilities. We speculate that the phenyl group of this compound might have functioned as the electron-donative property and/or bulky moiety and that it is necessary for the tight recognition of ALR2.

To understand the structural basis of the interaction of **15** with ALR2, we built a docking model of the ALR2–**15** complex using Glide 5.0 (Schrödinger, LLC.). Protein–ligand docking was performed by the following methods. First, we collected 52 ALR2 structures deposited in PDB and selected eight representative structures (PDB code: 2FZB, 2FZD, 2INE, 2NVC, 2NVD, 2PDG, 2PFH, 2PZN) as targets for docking based on the ligand binding site comparison. Next, we performed docking of **15** with the eight representative structures using Glide 5.0 in standard precision mode. Finally, the generated poses were ranked according to the Glide score and the top scoring pose

was selected as a predicted binding pose. Figure 2A shows the docking model of the ALR2–**15** complex. The lactone ring of coumarin form hydrogen bonds to the main chain NH group of Ala299 and Leu300. The C7 OH group of coumarin forms a hydrogen bond to Cys303, and the C6 OH group of coumarin forms a hydrogen bond to Cys80. The experimental data indicates that the C7 OH group and the C6 OH group enhance ALR2 inhibition activity, and the docking model supports the experimental result. Furthermore, aromatic residues in the ligand binding site seem to contribute to ALR2–**15** interactions. Coumarin scaffold forms π-π stacking to Trp111 and the C4 phenyl group forms vdW interactions with Trp20, Tyr48, Trp79, His110 and Phe122. The experimental data of **15** and **7** indicates that the phenyl group enhances ALR2 inhibition activity and the docking model can explain the experimental result well. Furthermore, Figure 2B shows the docking model of the ALR2-epalrestat complex. The docking study shows that the phenyl group of epalrestat interacts with Phe122 and it does not form π–π stacking with Trp111 which is observed in the ALR2–**15** complex. This result suggests that epalrestat interacts with ALR2 in a different binding mode from **15** and these phenylcoumarins appear to have potential as novel ALR2 inhibitors.

The experimental data of **15** and **12** shows that the C6 OH group is important for the selectivity towards ALR2. We compared the ligand binding site of ALR1 with ALR2 and discuss the structural importance for selectivity. Eight residues gave different results for ligand binding to ALR1 and ALR2 (Table 2). The C6 OH group forms hydrogen bonds to Cys80 in ALR2 and Asn83 in ALR1, respectively (Fig. 3). Both enzymes appear to interact reasonably with **15**, it is difficult to explain the selectivity by the simple docking study. More accurate calculation (e.g., MD simulation or binding free energy calculation) or X-ray crystal structure analysis of ALR1/ALR2 with **15** would enable a better understanding the selectivity.

The accumulation of polyols such as sorbitol or galactitol are thought to be responsible for development of cataracts.[21] In order to estimate the ex vivo effect of 6,7-dihydroxy-4-phenylcoumarin (**15**), we examined the inhibitory effect on the accumulation of galactitol in the rat lens according to the methods described in our recent paper.[22] Lens changes occur more rapidly under galactosemic conditions because glucose is converted to fructose by ALR2 and SDH in the sorbitol pathway but galactitol is not further metabolized by SDH. As shown by the results in Figure 4, 6,7-dihydroxy-4-phenylcoumarin (**15**) clearly suppressed the accumulation of galactitol in a dose-dependent manner.

The present work demonstrates that 6,7-dihydroxy-4-phenylcoumarin (**15**) has selective inhibition activity to ALR2.

Table 2
Amino acid sequences comparison of ALR1 and ALR2 in the ligand binding sites

ALR2		ALR1	
W	20	W	22
V	**47**	**I**	**49**
Y	48	Y	50
W	79	W	82
C	**80**	**N**	**83**
H	110	H	113
W	111	W	114
T	**113**	**Y**	**116**
F	115	F	118
F	122	F	125
W	219	W	220
V	**297**	**Y**	**298**
C	**298**	**I**	**299**
A	**299**	**V**	**300**
L	**300**	**P**	**301**
C	**303**	**D**	**313**
Y	309	Y	319
P	310	P	320
F	311	F	321

Residues that differ between ALR2 and ALR1 are indicated in bold.

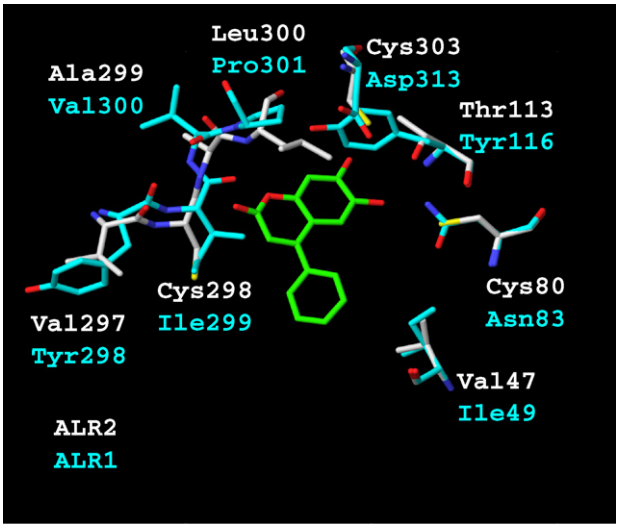

Figure 3. Structural comparison of ALR2 (white) and ALR1 (cyan) in ligand binding sites.

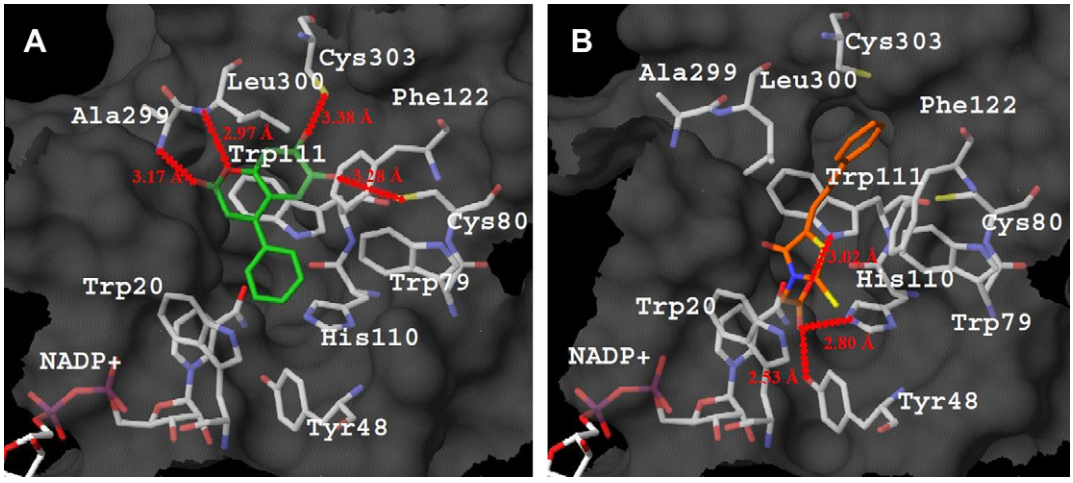

Figure 2. Docking model of ALR2 with 6,7-dihydroxy-4-phenylcoumarin (**15**: A) and epalrestat (B).

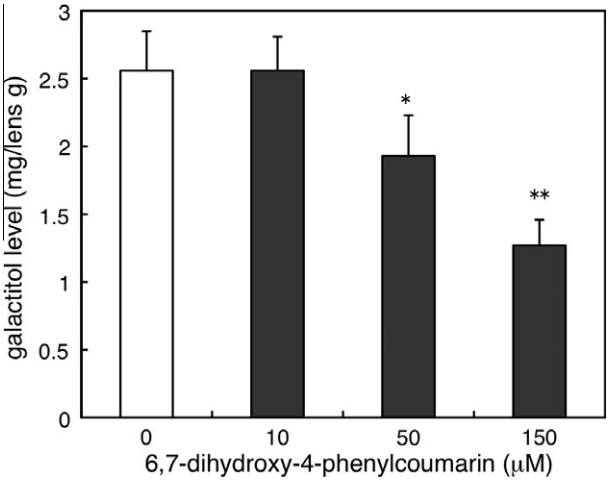

Figure 4. Effects of 6,7-dihydroxy-4-phenylcoumarin (**15**) on galactitol accumulation in rat lens. Each value represents the mean ± SEM ($n = 3$). **: Significant difference ($p < 0.01$) and *: Significant difference ($p < 0.05$) compared with non-treated.

Structure–activity studies revealed that the C7 OH group is an essential feature for recognition of ALR2 and the C6 OH group influences its ALR2 selectivity. Moreover, the C4 phenyl group contributes to improve the selectivity and potency toward ALR2. Our molecular docking study suggested that **15** binds to ALR2 in a different manner from epalrestat. Further studies are underway but these 4-phenylcoumarins appear to have great potential for developing a new-type of selective ALR2 inhibitor.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.038.

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