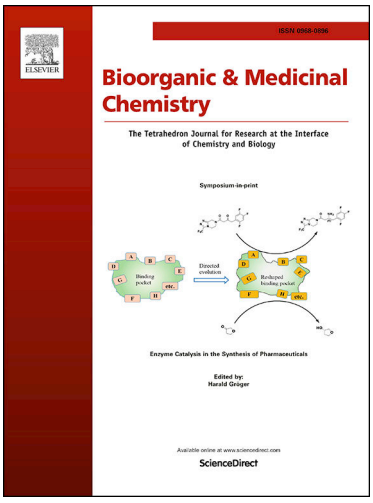
Journal Pre-proofs



Dihydrobenzoxazinone derivatives as aldose reductase inhibitors with antioxidant activity

Huan Chen, Xin Zhang, Xiaonan Zhang, Zhenya Fan, Wenchao Liu, Yanqi Lei, Changjin Zhu, Bing Ma

PII: S0968-0896(20)30529-0
DOI: https://doi.org/10.1016/j.bmc.2020.115699
Reference: BMC 115699

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 8 April 2020
Revised Date: 2 July 2020
Accepted Date: 4 August 2020

Please cite this article as: H. Chen, X. Zhang, X. Zhang, Z. Fan, W. Liu, Y. Lei, C. Zhu, B. Ma, Dihydrobenzoxazinone derivatives as aldose reductase inhibitors with antioxidant activity, *Bioorganic & Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.bmc.2020.115699

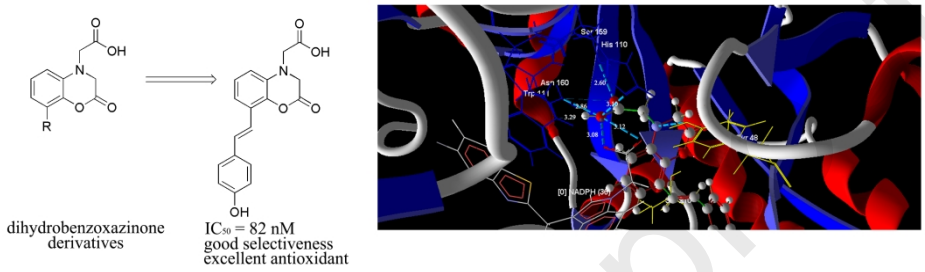
Graphical Abstract

Dihydrobenzoxazinone derivatives as aldose reductase inhibitors with antioxidant activity

Leave this area blank for abstract info.

Huan Chen, Xin Zhang, Xiaonan Zhang, Zhenya Fan, Wenchao Liu, Yanqi Lei, Changjin Zhu*, Bing Ma*.
Beijing Institute of Technology, No. 5, Zhongguancun South Street, 100081 Beijing, China

Aldose reductase inhibitors:



ELSEVIER

Bioorganic & Medicinal Chemistry
journal homepage: www.elsevier.com

Dihydrobenzoxazinone derivatives as aldose reductase inhibitors with antioxidant activity

Huan Chen [a], Xin Zhang[a], Xiaonan Zhang[a], Zhenya Fan[a], Wenchao Liu[a], Yanqi Lei[a], Changjin Zhu[a,*], Bing Ma [a,*].

[a]*School of Chemistry and Chemical Engineering, Beijing Institute of Technology, No. 5, Zhongguancun South Street, 100081 Beijing, China.*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Aldose reductase inhibitors
Antioxidant activity
Dihydrobenzoxazinone derivatives
Diabetic complications

ABSTRACT

Dihydrobenzoxazinone based design and synthesis produced two series of compounds as aldose reductase (ALR2) inhibitor candidates. In particular, phenolic residues was embodied into the compounds for the combination of strengthening the inhibitory acitvity and antioxidant ability to retard the progression of diabetic complications. Most of the derivatives with styryl side chains exhibited excellent activities on selective ALR2 inhibition with IC_{50} values ranging from 0.082 to 0.308 μM, and {8-[2-(4-hydroxy-phenyl)-vinyl]-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl}-acetic acid (**3a**) was the most potent. More significantly, most of dihydrobenzoxazinone compounds revealed not only good inhibitory effect on ALR2, but also showed powerful antioxidant activity. Notably, phenolic compound **3a** was even comparable to well-known antioxidant Trolox, confirming that the C8 p-hydroxystyryl substitution was determinative structure of alleviating oxidative stress. Therefore, these results provided an achievement of multifunctional ALR2 inhibitors possessing both capacities for ALR2 inhibition and as antioxidants.

* Corresponding author. E-mail: mabing@bit.edu.cn (B. Ma), zcj@bit.edu.cn (C. Zhu)

Diabetes mellitus (DM) is a metabolic abnormality caused by different elements such as genetic factors, immune disorders, microbial infections, and their toxins, free radical toxins, and mental factors, leading to chronic complications, including neuropathy, nephropathy, and retinopathy, associated with long-term damage, dysfunction, and failure of different organs.[1,2] Accumulation of sorbitol in the polyol pathway of glucose metabolism and oxidative stress under the condition of high-level blood sugar are regarded as the major pathogenesis of diabetic complications.[3-5]

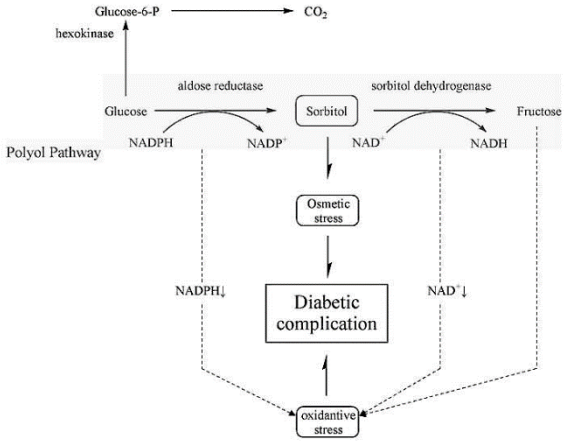


Figure 1. Metabolic pathway of glucose and pathogenesis of diabetic complications.

Aldose reductase (ALR2, EC 1.1.1.21) that is a member of the aldo-keto reductase superfamily is the first rate-determining enzyme of the polyol pathway (**Figure 1**).[6,7] Due to a relatively low affinity of ALR2 for glucose, only a small amount of substrate is metabolized through the polyol pathway. Most of the glucose is phosphorylated by hexokinase to form glucose 6-phosphate for glycolysis and the pentose phosphate pathway, which is a metabolic pathway parallel to glycolysis.[8,9] Under hyperglycemia conditions, however, hexokinase is rapidly saturated, and excess glucose is then metabolized by the polyol pathway, in which ALR2 reduces glucose to sorbitol utilizing NADPH as a cofactor.[10,11] The strong polarity of sorbitol hinders easy penetration through membranes and subsequent removal from tissues by diffusion.[12] Since sorbitol is formed faster than it is converted to fructose, it will be accumulated directly through the stimulated polyol pathway, and eventually leading to osmotic imbalance, cell swelling, and membrane permeability changes.[2] Also, both the exhaustion of NADPH and the disturbance of the NADH/NAD^+ ratio alter cellular redox potentials. This disorders impair the activity of enzymes such as nitric oxide synthase (NOS) and glutathione reductase, and further leading to cellular oxidative stress, as a consequence of the imbalance between increased production of radical oxygen species (ROS) and reduced intracellular antioxidant defense.[13] Furthermore, connected with the polyol pathway activation, the increased level of fructose enhances the intracellular formation of advanced glycation end products which results in further generation of ROS.[14] Obviously, both osmotic stress and oxidative stress induced by enhancement of ALR2 are responsible for cellular damage associated with diabetic complications. Therefore, the ALR2 inhibition and is supposed to be an efficient way to remedy and delay the development of diabetic complications.[15]

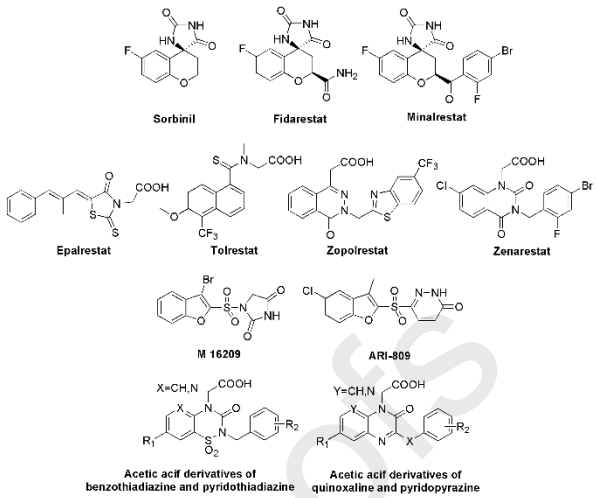


Figure 2. Structures of representative aldose reductase inhibitors.

By restraining the abnormal accumulation of sorbitol and decreasing the level of oxidative stress, ALR2 inhibitors (ARIs) are attractive agents preventing and alleviating diabetic complications.[16-18] Since the early 1970s, a large variety of synthetic ARIs have been developed, and some of them have been evaluated in preclinical and clinical trials (**Figure 2**).[19-24] There are three major categories according to their functional groups; spirohydantoin derivatives, sulfonyl derivatives, and carboxylic acid derivatives. Spirohydantoin inhibitors such as sorbinil and fidarestat were effective but resulted in hypersensitivity and hepatotoxicity as side effects.[24,25] New classes of sulfonyl ARIs including ARI-809 and M-16209 have been reported for their excellent selectivity and potent inhibitory activity both *in vitro* and *in vivo*.[26,27] However, the efficacy of sulfonyl derivative in clinic trials remained unknown. Comparing most reported inhibitors, the carboxylic acid derivatives emerged as potent ARIs, in which epalrestat has been identified as a highly potent ARI with a favorable pharmacokinetic profile and is the only commercial one for treatment of diabetic complications.[29,30] Aside from epalrestat, other carboxylic acid ARIs have failed in clinical trials because of pharmacokinetic drawbacks, adverse side effects, or low efficacy. Poor selectivity over aldehyde reductase (ALR1, EC 1.1.1.2), which plays a detoxification role in specifically metabolizing toxic aldehydes, is believed to be the crucial reason for the side effects.[31-33] Both ALR2 and ALR1 belong to the aldo-keto reductase superfamily, sharing structural homology with the difference at the C-terminal end of the enzyme proteins, which is the region lining the hydrophobic pocket of the active site called the 'specificity pocket' and responsible for specificity of inhibitors between the two enzymes.[34,35]

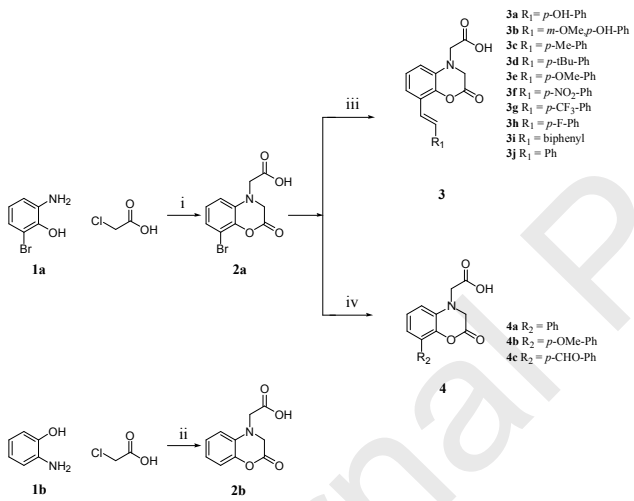
Thus, the design and synthesis of multifunctional ARIs may be a new efficient strategy to selectively inhibit ALR2 and to simultaneously retard the process of oxidative stress. Synthetic benzoxazinones have demonstrated potential for the development of new medications for treating diseases, and some of them showed interesting activity of antioxidant.[36-39] Besides, benzoxazinone derivatives were identified as potential neuroprotective agents possessing the antioxidant effect comparable to α-tocopherol.[38] Notably, isopropyl-substituted analogs have been tested in ALR2 inhibitory assay but showed

wea
1 μM).[39] In the present study, a new series of substituted 3,4-dihydro-(2H)-1,4-benzoxazin-2-one derivatives were designed to verify their ALR2 inhibition, selectivity, and antioxidant effect.

2. Result and discussion

2.1. Chemistry

The syntheses of all title compounds with an acetic acid substituent at N4 position and a variety of aromatic residues at C8 position of dihydrobenzoxazinone scaffold followed the general route outlined in **Scheme 1**. (8-Bromo-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl)-acetic acid (**2a**) as a key intermediate was obtained by the N-alkylation of 2-amino-6-bromophenol with 2-chloroacetic acid and then intramolecular condensation. **2b** without substituent at the C8 position of the scaffold was prepared from 2-aminophenol and 2-chloroacetic acid by a similar method. Different styrenes were readily attached to the C8 position of **2a** by Heck crossing coupling to give C8-styryl derivatives (**3a-j**). Subjected to Suzuki coupling reaction with the corresponding phenylboronic acid, C8-phenyl compounds (**4a-c**) were then obtained.

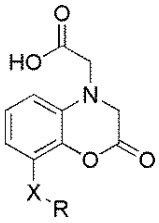


Scheme 1. Reagents and conditions: (i) NaOH, H_2O 100 °C, 4 h, 42%; (ii) NaOH, H_2O 100 °C, 3 h, 56%; (iii) styrene, $Pd(OAc)_2$, $P(o\text{-}tolyl)_3$, Et_3N, DMF, 110 °C, 32~41%; (iv) $C_6H_5B(OH)_2$, K_2CO_3, $Pd(OAc)_2$, *i*-PrOH, H_2O, 100 °C, 12~26%.

All benzoxazinone derivatives were screened for their inhibitory effect on ALR2 isolated from rat lenses. Only significantly active compounds coming from the test for the ALR2 inhibition were then subjected to estimate for their inhibitory activity against ALR1 isolated from rat kidneys, in order to evaluate their selectivity for ALR2. Epalrestat was utilized as a positive ARI to ensure validity. The results were summarized in **Table 1**.

In the ALR2 inhibition, compounds with the C8 side chain were more efficient (**3a-j**, **4a-c**) than the compound having no such side chains (**2b**). There were two series of C8-substituted compounds obtained according to the spacer length between the C8 position and the aromatic ring of the side chain. The first series of compounds contained styryl residues (**3a-j**) possessed a two-atom length spacer, while in the other series (**4a-c**) phenyl rings were directly attached to the C8 position. Compounds in the first series showed good inhibitory activity with IC_{50} values varying from 0.082 to 0.308 μM, which was much stronger than the counterparts in the second series with IC_{50} values ranging between 0.774 and 3.34 μM. Of all, compound **3a** embodied with the C8-vinylphenolic group showed the most potent inhibitory effect (IC_{50} = 0.082 μM) which is close to that of epalrestat, the positive control. Besides, phenolic compound **3b** also had excellent activity (IC_{50} = 0.188 μM), demonstrating that the introduction of phenolic group could reinforce the inhibitory activity. Among the styryl series, the inhibitory activity of all compounds was increased by substituents of alkyl, heteroalkyl, hetero, and halogen at the aromatic residue of the styryl side (**3a-i** > **3j**). Interestingly, with increased hydrophobicity of the alkyl group at C8-styryl residues, compounds were more active against ALR2 (**3d** > **3c** > **3j**). In addition, the compounds with electronic-donating groups or electronic-withdrawing groups at the aromatic ring of the C8-styryl were active in the ALR2 inhibition, implying a positive effect by altering the electron density of C8 aromatic rings.

The synthetic products with potent activity of ALR2 inhibition (IC_{50} < 0.200 μM), as well as compounds **3j** and **2b** for comparisons, were tested for their inhibition against ALR1 in order to evaluate their selectivity. Most of these compounds were inactive, demonstrating their inclination of the ALR2 inhibition. Of them, compound **3a** had the highest selectivity towards ALR2 over ALR1 with the selectivity index (SI) of 60.58, revealing that **3a** was superior to eparlestat. Notably, the SI values were decreased by alkyl substitution yet increased by polar residues.



Comp.	Substit. R	Substit. X	IC_{50} for ALR2 (μM) [a]	IC_{50} for ALR1 (μM) [a]	Selectivity index [b]
3a	*p*-OH-Ph	CH=CH	0.082±0.011	4.992±0.345	60.58
3b	*m*-OMe,*p*-OH-Ph	CH=CH	0.188±0.017	4.498±0.507	23.96
3c	*p*-Me-Ph	CH=CH	0.220±0.097		
3d	*p*-tBu-Ph	CH=CH	0.170±0.064	0.669±0.333	3.93
3e	*p*-OMe-Ph	CH=CH	0.208±0.082		
3f	*p*-NO_2-Ph	CH=CH	0.262±0.069		
3g	*p*-CF_3-Ph	CH=CH	0.138±0.047	3.268±0.214	23.68
3h	*p*-F-Ph	CH=CH	0.167±0.018	4.280±0.219	25.66
3i	biphenyl	CH=CH	0.281±0.018		
3j	Ph	CH=CH	0.308±0.067	2.812±0.097	9.11
4a	Ph	-	3.34±0.71		
4b	*p*-OMe-Ph	-	0.774±0.054		
4c	*p*-CHO-Ph	-	1.05±0.31		
2b	H	-	1.932±0.421	13.72±1.552	7.10
Epalrestat			0.045±0.010	1.198±0.034	26.70

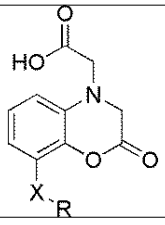
[a] n = 3.
[b] Defined as IC_{50}[ALR1] : IC_{50}[ALR2].

2.3. DPPH radical scavenging activity

Oxidative stress plays a vital role in the pathological process of diabetic complications. To decrease the level of oxidative stress and alleviate the disorder, ARIs with antioxidant capacities were desirable. Thus, the antioxidant functions of the compounds being active in the ALR2 inhibition were investigated.

The free radical scavenging capacity of the compounds was measured by a model reaction with the stable free radical of 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH), and 6-hydroxy-2,5,7,8-chroman-2-carboxylic acid (Trolox) served as a reference compound. Several dihydrobenzoxazinone derivatives which had good selectivity in ALR2 inhibitory (**3a-b**, **3g**, **3h**) were tested for DPPH radical scavenging activity, and compounds **3j**, **4a** and **2b** served as comparisons. Derivatives **3a-b**, **3h**, **3j**, which were better than Trolox, showed excellent DPPH radical scavenging activity (**Table 2**). Among them, compound **3b** had attractive antioxidant properties, achieving 18.22% of inhibition rate at a concentration of 1 μM. It should also be pointed out that **3a-b** were more effective than **3j**, suggesting that the phenolic subunit might be a structural determinant of DPPH free radical scavenging activity. What is more, compounds **3a-b**, **3g**, **3h,** and **3j** also exhibited better antioxidant activity than that **4a** and **2b** did, representing that styryl side chains contributed to the elimination of free radicals.

Table 2. DPPH radical scavenging activity of dihydrobenzoxazinone derivatives



Comp.	Substit.	% of inhibition of control value [a]

3a	*p*-OH-Ph	CH=CH	93.25±1.32	87.55±1.19	57.36±1.10	14.72±1.62
3b	*m*-OMe,*p*-OH-Ph	CH=CH	93.09±2.96	84.98±4.53	57.77±0.75	18.22±4.81
3g	*p*-CF_3-Ph	CH=CH	91.34±0.61	89.18±0.81	44.30±1.03	9.92±0.79
3h	*p*-F-Ph	CH=CH	92.64±0.31	90.04±0.31	57.14±1.06	12.55±1.62
3j	Ph	CH=CH	86.50±2.33	84.18±1.03	43.63±3.11	12.03±1.86
4a	Ph	-	91.35±0.60	36.5±1.66	13.24±2.31	6.12±0.79
2b	H	-	92.83±2.13	65.61±1.64	23.14±1.73	7.17±1.19
Trolox			94.62±0.29	82.61±3.96	47.82±1.34	10.17±1.79

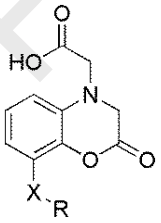
[a] n = 3

2.4. Lipid peroxidation suppression

Generating from the reaction of thiobarbituric acid with malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS) are regarded as an index of lipid peroxidation. Induced by radical oxidation, the lipoperoxidation product of MDA will finally result in dramatic damage to cellular membranes and proteins. In order to further identify whether the compounds described above can act as the antioxidants through a different mechanism in an oxidative pathway, we also examined the effect of compounds on the hydroxyl radical-dependent lipoperoxidation in rat brain homogenate induced by the oxidant system Fe(III)/ascorbic acid.[40,41]

Compounds **3a-b, 3h, and 3j** that were already active in the DPPH radical scavenging activity were evaluated in the suppression of the lipid peroxidation. Compounds **3j** and **2b** served as comparisons. Consistent with the conclusion of the DPPH radical scavenging assay, inhibitory data of the lipid peroxidation showed that tested compounds bearing styryl side chains (**3a-b**, **3h**, **3j**) were active in the indicative heterogeneous assay (**Table 3**). The activities of these suppressors were equivalent to that of Trolox. Of them, compounds **3a-b** had appreciable antioxidant properties and were more effective than the other tested compounds. This indicated the importance of the phenolic substituents in the derivatives for their antioxidant activity.

Table 3. Inhibition data of lipid peroxidation for benzoxazinone derivatives



Comp.	Substit.		MDA inh. % [a]
	R	X	100μM
3a	*p*-OH-Ph	CH=CH	81.70±1.22
3b	*m*-OMe,*p*-OH-Ph	CH=CH	80.72±0.92
3h	*p*-F-Ph	CH=CH	74.84±0.46
3j	Ph	CH=CH	75.82±1.22
2b	H	-	72.22±1.67
Trolox			75.49±2.40

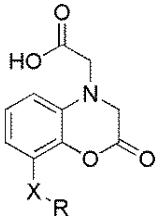
[a] n = 3

2.5. Scavenging activity of superoxide anion radicals

various diseases such as cancer, cardiovascular diseases, immune system decline, and diabetes. Under diabetic conditions, various sources of superoxide are activated, including xanthine oxidases, NADPH oxidases, uncoupling of nitric oxide synthase, and mitochondrial fuel oxidative processes. As a precursor of ROS, superoxide species are overproduced and accumulated, resulting in damage to cellular components.

provided with excellent antioxidant effect in both the tests of DPPH radical and MDA. Similarly, they exhibited good activity suppressing superoxide anion radicals, and were comparable to a reference compound of Trolox (**3a** ≈ **3b** ≈ Trolox) (**Table 4**). Unfortunately, the activities of compounds **3h**, **3j,** and **2b** were much lower than that of Trolox. Again, these results are explicit that compounds with phenolic styryl group had potential in the removal of superoxide radicals, preventing the overproduction of ROS.

Table 4. Superoxide anion radicals scavenging activity of dihydrobenzoxazinone derivatives



Comp.	Substit. R	Substit. X	$O_2^{\bullet-}$ inh. activity (U/*u*M) [a]
3a	*p*-OH-Ph	CH=CH	1.07±0.01
3b	*m*-OMe,*p*-OH-Ph	CH=CH	1.02±0.03
3h	*p*-F-Ph	CH=CH	0.79±0.15
3j	Ph	CH=CH	0.81±0.04
2b	H	-	0.73±0.03
Trolox			1.13±0.01

[a] n = 3

By the combination of the DPPH radical clearance, scavenging of the superoxide anion radicals, and the MDA suppression, dihydrobenzoxazinone derivatives have been proved to work as potent antioxidants even compared favorably with the typical antioxidant agent of Trolox. These results also revealed that phenolic substitutions of the derivatives (**3a-b**) were essential for the ideal antioxidant property of these ARIs.

2.6. Molecular docking

To better understand the binding details in the ALR2 inhibition of C8-styryl dihydrobenzoxazinone derivatives, molecular docking of compounds **3a**, **3d-e**, and **3g-j** possessing significant activity in the ALR2 inhibition were performed. Compounds **4a** and **2b** that were less active in the ALR2 inhibition were also docked. The conformation resulted from the ligand of lidorestat was selected for the docking study because structures of compounds we potently identified were close to the ligand more than other ligands. When docking of compounds into the human ALR2/$NADP^+$/lidorestat complex (PDB code: 1Z3N),[42] **3a**, **3d-e, 3g-j** and lidorestat bound well to the active site of ALR2 (**Fig. 3**). The carboxyl group of these compounds were inserted deeply into the anion binding pocket by hydrogen bonds interacting. However, the carboxyl group of compounds **2b** totally "flowed out" of the anion binding pocket. Moreover, the lengths of hydrogen bonds suggest that compounds **3a**, **3d-e**, and **3g-j** bound to the active site much more tightly than **4a** indicating the good inhibitory activity of the C8-styryl compounds. Besides, the lengths of hydrogen bonds formed by **3a** and **3j** in the anion binding pocket suggest that **3a** might be closer to the activate site than **3j**. This was coincident with the results from the ALR2 inhibition, in which **3a** was more potent than **3j**. Surprisingly, according to docking results displayed in **Fig. 3** and **Table 5**, hydrogen bonds between the carboxyl group of lidorestat and amino residues located in anion site were weaker than that of styryl type compounds.

Moreover, the binding energy for **3a**, **3d-e**, **3g-j** and **4a** was calculated (**Table 5**) to further evaluate their inhibitory efficiency. Data for **2b** was excluded since its carboxyl group failed in the binding to the anion pocket. All of the binding free energy for **3a**, **3d-e**, and **3g-j** were lower than that for **4a**, illustrating better affinities of C8-styryl derivatives for ALR2.

Am DP+ demonstrating the highest binding affinity and inhibitory activity. **3g** also showed a significant interaction with ALR2. These results were consistent with that derived from the ALR2 inhibition. Notably, both hydrogen bonds energies of **3d** and **3e** were lower than that of **3a**, yet the binding affinity scores were higher. We consumed that the interactions might be affected by steric hindrance of the alkyl groups located in aromatic ring of C8-substituents. Although lidorestat displayed excellent inhibition against ALR2 (IC_{50} = 5 nM)[43], the docking energy result revealed that lidorestat stabilized weak hydrogen bond interaction with the enzyme.

complex (PDB code: 1HQ1)[44] was also investigated to illustrate the selectiveness towards ALR2 over ALR1. The total energies of compounds **3a**, **3d**, **3g-h**, **3j** and lidorestat were calculated and presented in **Table 6**. Remarkably, consistent with the results in ALR1 inhibitory experiment, **3a** exhibited highest reranked score among all the tested derivatives, proving the best selectivity of **3a** for ALR2. Besides, the energy value of ligand **3d** was much lower than that of **3j,** explaining the result that alkyl group located in aromatic ring of C8-substituents had a negative effect on the selectivity.

Table 5. MolDock and reranked score for dihydrobenzoxazinone ligands against ALR2

	Total Energy		
Ligand	MolDock Score (Kcal/mol)	Rerank Score (Kcal/mol)	Hydrogen bonds (Kcal/mol)
3a	-172.130	-143.031	-8.726
3d	-154.254	-101.134	-13.402
3e	-138.379	-96.155	-10.697
3g	-173.017	-142.519	-6.578
3h	-137.533	-97.708	-5.895
3i	-157.412	-97.966	-4.274
3j	-133.745	-95.392	-6.487
4a	-130.337	-70.004	-6.705
lidorestat	-174.725	-134.905	-5.000

Table 6. MolDock and reranked score for dihydrobenzoxazinone ligands against ALR1

	Total Energy		
Ligand	MolDock Score (Kcal/mol)	Rerank Score (Kcal/mol)	Hydrogen bonds (Kcal/mol)
3a	-124.241	-68.757	-6.192
3d	-132.956	-106.772	-7.609
3g	-124.075	-97.454	-3.867
3h	-120.769	-83.952	-4.544
3j	-119.012	-98.323	-7.778
lidorestat	-140.112	-109.160	-7.312

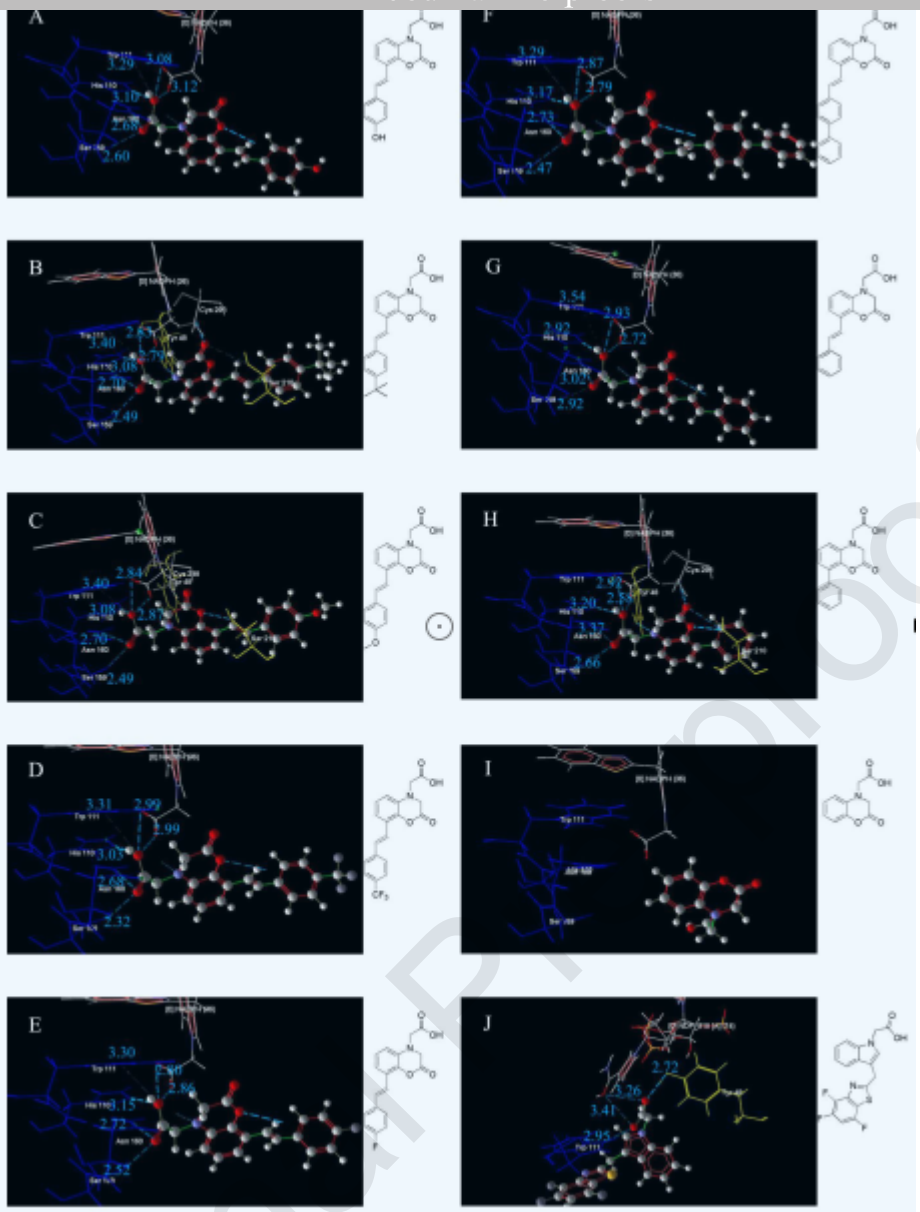


Figure 3. Docking of dihydrobenzoxazinone derivatives **3a** (A), **3d** (B), **3e** (C), **3g** (D), **3h** (E), **3i** (F), **3j** (G), **4a** (H), **2b** (I) and lidorestat (J) into the active site of ALR2. The docked poses of the compounds are shown in white (C), red (O) and blue (N). Hydrogen bonds are shown as blue dashed lines, and lengths (Å) of the hydrogen bonds are labeled beside the dashed lines

3. Conclusions

In this paper, we designed and synthesized a series of dihydrobenzoxazinone based derivatives by altering of the C8 side chain to improve these ALR2 inhibitors. In general, to strengthen the efficacy of retarding the long-term complications of diabetes, compounds were designed for a combination of ALR2 inhibition

activ... 532, **3** with styryl residues showed a good inhibitory activity and selectivity towards ALR2, and **3a** was verified as the most potent. More significantly, **3a-b** and **3g-h** not only readily inhibit ALR2 with remarkable selectivity but also exhibited excellent activities in DPPH radical scavenging, lipid peroxidation suppression and superoxide anion radical's clearance. Notably, containing with phenolic group in the C8-styryl side chain, **3a-b** were even superior to the conventional antioxidant Trolox. The results derived from structure-activity relationship analysis combined with further docking studies demonstrated that the substitution of C8-styryl side chain had a positive effect on the inhibitory activity and the selectivity, and introduction of *p*-hydroxyl to the aromatic rings of the side chains reinforced both the inhibitory activity and the antioxidant property. As a consequence, with a structural determinant of C8 *p*-hydroxystyryl side chain, dihydrobenzoxazinone compound **3a** could serve as a significant lead for the achievement of multifunctional ALR2 inhibitors.

4. Experiment

4.1. Chemistry

Melting points were recorded on an X-4 microscopic melting point apparatus and are uncorrected. All reactions were routinely checked by TLC on silica gel Merck 60F254. The NMR spectra were recorded on a Bruker Ascend 400 M spectrometer (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) and chemical shifts were given in δ units (ppm) relative to internal standard TMS and refer to DMSO-*d6* solutions. HRMS (ESI) was performed using an AGILENT LC/MS. Analysis of sample purity was performed on a Hitachi D-2000 Elite HPLC system. HPLC conditions were the following: Inertsil ODS-2 250 mm × 10 mm, 5 mm column; mobile phase: CH_3CN (0.1% TFA) /CH_3OH = 1/1, for 10 min; room temperature; flow rate: 1 mL min^{-1} ; detection at λ 254 nm. All final compounds in biological assays have a purity of ⩾95%.

*4.2. Procedure for preparation of (8-Bromo-2-oxo-2,3-dihydro-benzo[1,4]oxazin -4-yl)-acetic acid (***2a***)*

A mixture of 6-bromo-2-aminophenol (8.82 g, 0.049 mol), chloroacetic acid (9.8 g, 0.1 mol), NaOH (6 g, 0.15 mol), in 100 mL water was stirred at 100 °C for 4 h under argon. A solution of NaOH (6 M, aq) was added dropwise to the reaction in order to maintain pH at 7–8. The reaction mixture was cooled and concentrated HCl was added until the pH of the solution was strongly acidic. Solid brown deposits were filtered and purified by silica gel column chromatography with petroleum ether/ethyl acetate (3:1) to give the desired compound **2a**, yield 42%.

4.2.1. (8-Bromo-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl)-acetic acid (**2a**)

Yield: 5.86 g (42%); brown solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 7.51 (s, 1H), 6.93 (m, 1H), 6.86 (d,J = 7.8 Hz, 1H), 4.20 (s, 2H), 4.16 (s, 2H).

*4.3. Procedure for preparation of (2-Oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl)-acetic acid (***2b***)*

Compound **2b** was prepared by similar method with 2-aminophenol, yield 56%.

4.3.1. (2-Oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl)-acetic acid (**2b**)

Yield: 5.68 g (56%); pink solid; m.p. 166-168 °C; purity: 98.92%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.92 (s, 1H), 7.21-7.18 (d, J = 7.8 Hz, 2H), 6.91 (m, 1H), 6.89 (d, J = 7.8 Hz, 1H), 4.19 (s, 2H), 4.14 (s, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.33, 165.04, 141.55, 134.72, 125.44, 119.85, 116.81, 113.56, found 206.0463 .

*4.4. General procedure for preparation of (2-Oxo-8-styryl-2,3-dihydro-benzo[1,4] oxazin-4-yl)-acetic acid (***3***)*

$Pd(OAc)_2$ (0.011 g, 0.05 mmol) and P(o-tolyl)$_3$ (0.030 g, 0.10 mmol) were added to a solution of **2a** (0.287 g, 1 mmol) in DMF (5 mL) and Et_3N (0.5 mL). After being stirred at room temperature under argon for 20 min, the appropriate styrene (1.2 mmol) was added. The reaction mixture was stirred at 110 °C for 14 h. After the completion of the reaction, the mixture was poured in NaOH solution and washed with 100 mL of ethyl acetate five times. The aqueous phase was collected and 6M HCl solution was added until pH at 2~3, then extracted by ethyl acetate three times to collect organic layer, dried with $MgSO_4$. After filtration and evaporation of ethyl acetate, the residue was purified by silica gel column chromatography with petroleum ether/ethyl acetate (3:1~1:1) to give desirable products **3**, yield 32~41%.

4.4.1. {8-[2-(4-Hydroxy-phenyl)-vinyl]-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl}-acetic acid (**3a**)

Yield: 0.111 g (34%); brown solid; m.p. 165-167 °C; purity: 98.17%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (s, 1H), 9.66 (s, 1H), 7.58 (m, 3H), 7.41(d, J = 2.1 Hz, 1H) 7.04 (m, 2H), 6.89 (d, J = 7.4 Hz, 1H), 4.74 (s, 2H), 4.51 (s, 2H). ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.94, 164.92, 158.01, 142.29, 130.65, 129.75, 129.49, 128.42, 126.74, 122.93, 120.61, 118.48, 116.12, 114.27, 52.23, 43.35 ppm. HRMS (ESI) m/z calcd. for [M-H]$^-$ 324.0950 , found 324.0889 .

4.4.2. {8-[2-(3-Methoxy-4-hydroxy-phenyl)-vinyl]-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl}- acetic acid (**3b**)

Yield: 0.128 g (36%); yellow solid; m.p. 156-157 °C; purity: 97.31%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (s, 1H), 9.32 (s, 1H), 7.29 (m, 1H), 7.21 (m, 3H) 7.09 (m, 2H), 6.74 (d, J = 7.4 Hz, 2H), 6.63 (d J = 2.3 Hz, 1H), 4.16 (s, 2H), 4.13 (s, 2H), 3.68 (s, 1H). ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.82, 165.00, 148.31, 147.42, 131.15, 129.70, 129.10, 126.80, 122.97, 118,76, 114.27, 110.53, 67.55, 60.22, 56.11 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 354.1056, found 354.0998 .

4.4.3. [2-Oxo-8-(2-p-tolyl-vinyl)-2,3-dihydro-benzo[1,4]oxazin-4-yl]-acetic acid (**3c**)

Yield: 0.132 g (41%); yellow solid; m.p. 125-127 °C; purity: 95.88%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.97 (s, 1H), 7.43 (d, J = 7.4 Hz, 2H), 7.41-7.20 (m, 5H) 7.09 (m, 1H), 6.74 (d, J = 7.4 Hz, 1H), 4.15 (s, 2H), 4.12 (s, 2H), 2.46 (s, 3H). ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.40, 164.87, 138.79, 137.95, 134.59, 129.77, 124.98, 120.32, 116.83, 112.62, 50.51, 50.15, 21.33 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 322.1158, found 322.1093.

4.4.4. {8-[2-(4-tert-Butyl-phenyl)-vinyl]-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl}-acetic acid (**3d**)

Yield: 0.128 g (35%); pink solid; m.p. 142-143 °C; purity: 96.21%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.21-12.83 (s, 1H), 7.51 (d, J = 7.4 Hz, 2H), 7.48 (d, J = 7.4 Hz, 2H), 7.41-7.32 (m, 3H) 7.11 (m, 1H), 6.71 (d, J = 7.4 Hz, 1H), 4.13 (s, 2H), 4.10 (s, 2H), 1.89 (s, 9H). ;^{13}C NMR (100 MHz, DMSO-d_6) δ 171.41, 164.87, 151.10, 138.80, 134.61, 130.97,124.98, 120.53, 116.82, 112.65, 50.56, 50.16, 34.84, 31.52 ppm. HRMS (ESI) m/z calcd for [M-H]- 364.1627, found 364.1554.

4.4.5. {8-[2-(4-Methoxy-phenyl)-vinyl]-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl}-acetic acid (**3e**)

Yield: 0.135 g (40%); white solid; m.p. 170-171 °C; purity: 95.46%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H), 7.52 (d,

J = 2H), 6.61 (d J = 2.3 Hz, 1H), 4.15 (s, 2H), 4.11 (s, 2H), 3.72 (s, 1H). ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.41, 164.90, 159.72, 138.68 135.22, 130.84, 128.43, 125.88, 124.96, 119.00, 116.71, 114.75, 112.35, 55.64, 50.50, 50.16 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 338.1107, found 338.1038.

4.4.6. {8-[2-(4-Nitro-phenyl)-vinyl]-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl}-acetic acid (**3f**)

Yield: 0.138 g (39%); white solid; m.p. 168-169 °C; purity: 97.33%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.83 (s, 1H), 8.22 (s, 2H), 7.91 (d, J = 2.5 Hz, 1H), 7.58 -7.49 (m, 2H), 7.38 (s, 1H) 7.12 (m, 1H), 7.10 (d, J = 7.4 Hz, 2H), 6.65 (d, J = 2.3 Hz, 1H), 2.93 (s, 2H), 2.87 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.32, 162.78, 146.81, 144.36 143.18, 141.60, 139.31, 134.78, 134.67, 127.84, 124.60, 124.57, 120.09, 116.88, 113.68, 52.22, 50.05 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 353.0852, found 353.0788.

4.4.7. {2-Oxo-8-[2-(4-trifluoromethyl-phenyl)-vinyl]-2,3-dihydro-benzo[1,4]oxazin-4-yl}-acetic acid (**3g**)

Yield: 0.121 g (32%); yellow solid; m.p. 213-215 °C; purity: 98.49%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.24-12.81 (s, 1H), 7.91 (d, J = 2.5 Hz, 1H), 7.85 -7.71 (m, 4H), 7.60 -7.42 (m, 2H), 7.33 -7.10 (m, 2H), 7.38 (s, 1H) 7.12 (m, 1H), 2.97 (s, 2H), 2.86 (s, 2H) ;^{13}C NMR (100 MHz, DMSO-d_6) δ 169.76, 164.90, 142.98, 129.79, 129.12, 127.87, 127.64, 127.54, 127.39, 127.35, 126.14, 125.66, 124.77, 123.07, 121.43, 60.22, 43.11 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 376.0875, found 376.0805.

4.4.8. {8-[2-(4-Fluoro-phenyl)-vinyl]-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl}-acetic acid (**3h**)

Yield: 0.108 g (33%); yellow solid; m.p. 169-170 °C; purity: 97.10%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.00-12.78 (s, 1H), 7.69 (d, J = 7.2 Hz, 3H), 7.15 -7.31 (m, 4H), 7.12 (t, J = 7.2 Hz, 1H), 7.12 (d, J = 2.5 Hz, 1H), 4.21 (s, 2H), 4.10 (s, 2H) ;^{13}C NMR (100 MHz, DMSO-d_6) δ 171.39, 164.81, 138.86, 135.25, 129.05, 128.91, 124.99, 121.35, 116.06, 112.82, 97.89, 50.54, 50.15 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 326.0907, found 326.0850.

4.4.10. [8-(2-Biphenyl-4-yl-vinyl)-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl]-acetic acid (**3i**)

Yield: 0.123 g (32%); red solid; m.p. 212-213 °C; purity: 95.18%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.22-12.81 (s, 1H), 7.82 - 7,69 (m, 7H), 7.51 (d, J = 7.2 Hz, 3H), 7.28 (d, J = 2.4 Hz, 2H), 7.21 (d, J = 2.3 Hz, 1H), 6.72 (d, J = 7.2 Hz, 1H), 4.33 (s, 2H), 4.15 (s, 2H) ;^{13}C NMR (100 MHz, DMSO-d_6) δ 171.40, 167.43, 164.84, 140.03, 138.90, 136.55, 135.28, 129.13, 127.50, 125.02, 121.48, 116.95, 50.55, 50.16 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 384.1314, found 384.1245.

4.4.12. (2-Oxo-8-styryl-2,3-dihydro-benzo[1,4]oxazin-4-yl)-acetic acid (**3j**)

Yield: 0.127 g (41%); white solid; m.p. 176-177 °C; purity: 98.49%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.53 (d, J = 7.2 Hz, 2H), 7.48 - 7.40 (m, 2H), 7.38 - 7.31 (m, 2H), 7.22 (d, J = 7.2 Hz, 2H), 7.10 (d, J = 2.3 Hz, 1H), 6.71 (d, J = 7.2 Hz, 1H), 4.31 (s, 2H), 4.22 (s, 2H) ;^{13}C NMR (100 MHz, DMSO-d_6) δ 171.40, 164.84, 138.89, 137.34, 135.25, 129.30, 127.06, 125.01, 121.40, 116.95, 112.83, 50.53, 50.15 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 308.1001, found 308.0941.

*4.5. General procedure for preparation of (2-Oxo-8-phenyl-2,3-dihydro-benzo[1,4]-oxazin-4-yl)-acetic acid (**4**)*

To 50 mL flask, $Pd(OAc)_2$ (0.035 g, 0.15 mmol), K_2CO_3 (0.415 g, 3 mmol), water (8 mL), appropriate phenylboronic acid (2.5 mmol) and **2a** (0.287 g, 1 mmol) in isopropanol (2 mL) solution r an atmosphere of argon, and then washed with ethyl acetate five times. The aqueous solution was obtained and the pH of the solution was adjusted at 2~3 by 6M HCl aqueous. The suspension was extracted by ethyl acetate three times, dried with $MgSO_4$, and filtered and concentrated under reduced pressure. The crude product was flashed by column chromatography(ethyl acetate/petroleum ether 1:5~1:1) to provide the products **4**, yield 12~26%.

4.5.1 (2-Oxo-8-phenyl-2,3-dihydro-benzo[1,4]oxazin-4-yl)-acetic acid (**4a**)

Yield: 0.045 g (16%); white solid; m.p. 141-143 °C; purity: 96.05%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.89 (s, 1H), 7.76 (d, J = 2.3 Hz, 1H), 7.54 (d, J = 7.5 Hz, 2H), 7.21 (d, J = 7.5 Hz, 2H), 6.88 (m, 2H), 4.22 (s, 2H), 3.87 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.94, 167.44, 164.01, 137.07, 136.02, 132.18, 131.99, 129.79, 129.71, 129.65, 129.55, 129.14, 128.66, 50.55, 50.03 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 282.0845, found 282.0782 .

4.5.2. [8-(4-Methoxy-phenyl)-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl]-acetic acid (**4b**)

Yield: 0.037 g (12%); pink solid; m.p. 191-192 °C; purity: 95.30%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (s, 1H), 7.62 (d, J = 7.2 Hz, 2H), 7.52 (d, J = 7.5 Hz, 1H), 7.47 (d, J = 2.6 Hz, 1H), 7.21 (d, J = 7.2 Hz, 1H), 6.97 (m, 2H), 4.85 (s, 2H), 4.62 (s, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.19, 159.38, 151.97, 137.47, 130.95, 129.98, 129.89, 122.34, 121.02, 114.44, 114.26, 114.15, 65.31, 55.63 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 312.0950, found 312.0890 .

4.5.3. [8-(4-Formyl-phenyl)-2-oxo-2,3-dihydro-benzo[1,4]oxazin-4-yl]-acetic acid (**4c**)

Yield: 0.080 g (26%); white solid; m.p. 154-156 °C; purity: 97.33%,^{1}H NMR (400 MHz, DMSO-d_6) δ 12.97 (s, 1H), 10.14 (s, 1H), 8.07 (d, J = 7.2 Hz, 2H), 7.86 (d, J = 2.6 Hz, 2H), 7.30 (m, 1H), 7.87 - 7.65 (m, 2H), 4.38 (s, 2H), 4.22 (s, 2H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.30, 171.37, 164.92, 143.03, 138.44, 135.61, 130.46, 128.29, 121.02, 113.82, 50.53, 50.19 ppm. HRMS (ESI) m/z calcd for [M-H]$^-$ 310.0794, [M+HCOO$^-$]$^-$ 356.0770, found 310.0752, 356.1169.

4.6. Enzyme assays

The ALR2 inhibition activity was tested in a reaction mixture containing 0.25 mL NADPH (0.10 mM), 0.25 mL sodium phosphate buffer (pH = 6.2, 0.1 M), 0.1 mL enzyme extracted from rat lens, 0.15 mL deionized water, and 0.25 mL D,L-glyceraldehyde (10 mM) as substrate in a final volume of 1 mL. Before adding to substrate, the reaction mixture was incubated at 30 °C for 5 min, then the substrate was added to start the reaction, which was monitored for 4 min. The ALR1 inhibition activity was performed at 37 °C in a reaction mixture containing 0.25 mL NADPH (0.12 mM), 0.25 mL enzyme extracted from rat kidney, 0.25 mL sodium phosphate buffer (pH=7.2, 0.1 M), and 0.25 mL sodium D-gluconate (20 mM) as substrate in a final volume of 1 mL. Before adding to sodium D-gluconate, the reaction mixture was incubated at 37 °C for 4 min, then the substrate was added to start the reaction, which was monitored for 4 min.

The inhibitory activities of the newly synthesized compounds against ALR2 and ALR1 were assayed by adding 5 μL of the inhibitor solution to the reaction mixture described above. All compounds were dissolved in dimethyl sulfoxide (DMSO), and the solutions were diluted with DMSO to desirable concentrations. To correct for the nonenzymatic oxidation of NADPH, the rate of NADPH oxidation in the presence of all of the reaction mixture

com was experimental rate. The compounds were tested at concentrations ranging from 100 μM to 10 nM. Most dose-response curves were generated using at least three concentrations of the compound with inhibitory activity between 20% and 80%, with three replicates at each concentration, and the IC_{50} values were calculated by least-square analysis of the linear portion of the log (dose) versus response curves ($r^2 > 0.95$). The experiments were performed in triplicate.

4.7. Antioxidant assays

4.7.1. DPPH radical scavenging activity

To investigate the antioxidant activity of the given compounds in a homogeneous system, an experiment based on the stable free radical DPPH scavenging rate was conducted. Briefly, 100 μL methanol solution of testing compounds (or the reference compound Trolox) with varies of concentrations was added into the tube containing 1 mL of DPPH methanol solution (0.025 mg/mL) and 1.9 mL of methanol, to obtain final concentrations of 100, 10, 5 and 1 μM. The absorbance of the stable DPPH radical in the methanol solution at 517 nm continually decrease in the presence of the tested compounds, as measured. And the percentage of DPPH radical scavenging was determined after steady state by the equation as shown below. The experiments were performed in triplicate.

$$\text{Percentage of DPPH scavenging(\%)} = (1-\frac{A_{sample}-A_{blank}}{A_{control}})\times 100\%$$

Sample: tested compound + DPPH + methanol
Blank: tested compound + methanol
Control: DPPH + methanol

4.7.2. Inhibition of lipid peroxidation

Fresh rat brain was isolated and crushed with ice-cold normal saline to prepare 25% brain homogenate. The homogenate was centrifuged for 10 min at a speed of 3000 rpm, and the supernatant was used for biochemical analyses. The MDA concentration in the supernatant was determined using a commercially available kit based on thiobarbituric acid (TBA) reactivity. The reaction mixture containing the title compounds (100 μM), $FeCl_3$ (0.02 μM), ascorbic acid (0.1 μM) and brain homogenate supernatant was incubated at 37 °C for 30 min. Then, after mixing TBA with the reaction mixture and centrifuging, a supernatant was obtained. MDA concentration tests in the supernatants were carried out following the manufacturer's instructions (Malondialdehyde assay kit (TBA method), Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Briefly, the working mixture of the kit was prepared by volume ratio of reagent 1 : reagent 2 : reagent 3 = 1: 30: 10. Supernatants (0.1 mL) were added into tubes containing working mixture (4 mL). After boiled in 95°C for 40 min, the tubes were iced and centrifuged (4000 rpm, 10 min). The developed red product of the resulting reaction was measured at 532 nm with a spectrophotometer. The experiments were performed in triplicate.

$$\text{Percentage of MDA clearance(\%)} = (1-\frac{A_{sample}-A_{blank1}}{A_{control}-A_{blank2}})\times 100\%$$

Sample: tested compound + brain homogenate
Blank1: tested compound + saline
Control: methanol + brain homogenate
Blank2: methanol + saline

4.7.3. Superoxide anion radicals scavenging activity

was monitored by manufacturer's instruction of inhibition and produce superoxide anion assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). To a tube, reagent and compounds were added by following order: reagent 1 (1 mL), title compound (0.05 mL), reagent 2 (0.1 mL), reagent 3 (0.1 mL), reagent 4 (0.1 mL). Resulting mixture was incubated at 37 °C for 40 min, and then chromogenic reagent (2 mL) was added into the tube. After standing for 10 min, the solution was measured at 550 nm with a spectrophotometer. The experiments were performed in triplicate. Activity of inhibition against superoxide anion radicals production

$$(\text{U}/\mu\text{mol}) = \frac{A_{control}-A_{\text{sample}}}{A_{control}-A_{blank}}\times 150 \div \text{C}_{\text{sample}}(\mu M)$$

Control: ascorbic acid (150 mg/L)
Sample: title compound
Blank: distilled water

4.8. Docking studies

Docking was performed using Molegro Virtual Docker (version 5.0) with the same protocol as described previously.[45] Energy data was calculated by Molegro Virtual Docker.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant no. 21272025). Furthermore, the authors would like to thank Analysis & Testing Center, Beijing Institute of Technology (China) for technical support for performing in the structural analysis of synthetic compounds.

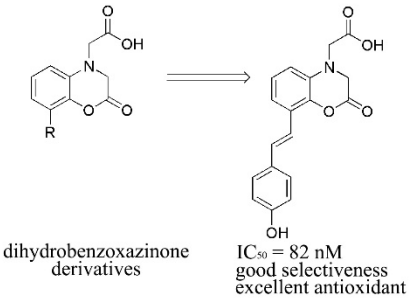
1. Cosconati, S.; Marinelli, L.; Motta, C. L.; Sartini, S.; Settimo, F. D.; Olson, A. J.; Novellino, E. *J. Med. Chem.* **2009**, *52*, 5578–5581. https://doi.org/10.1021/jm901045w.
2. Gabbay, K. H.; Merola, L. O. R.; Field, A. *Science*. **1966**, *151*, 209–210. https://doi.org/10.1126/science.151.3707.209.
3. Srivastava, S. K.; Ramana, K. V.; Bhatnagar, A. *Endocr. Rev.* **2005**, *26*, 380–392. https://doi.org/10.1210/er.2004-0028.
4. Alexiou, P.; Pegklidou, K.; Chatzopoulou, M.; Nicolaou, I.; Demopoulos, V. J. *Curr. Med. Chem*. **2009**, *16*, 734–752. https://doi.org/10.2174/092986709787458362.
5. Brownlee, M. *Diabetes*, **2005**, *54*, 1615–1625. https://doi.org/10.2337/diabetes.54.6.1615
6. Kinoshita, J. H.; Nishimura, C. *Diabetes/Metab. Rev.* **1988**, *4*, 323-337. https://doi.org/10.1002/dmr.5610040403.
7. Chung, S. S.; Chung, S. K. *Curr. Med. Chem.* **2003**, *10*, 1375-1387. https://doi.org/10.2174/0929867033457322.
8. Hers, H. G. *Biochim. Biophys. Acta.* **1956**, *22*, 202-203. https://doi.org/10.1016/0006-3002(56)90247-5.
9. Suzen, S.; Buyukbingol, E. *Curr. Med. Chem.* **2003**, *10*, 1329-1352. https://doi.org/10.2174/0929867033457377.
10. Gabbay, K. H. *N. Engl. J. Med.* **1973**, *288*, 831-836. https://doi.org/10.1056/NEJM197304192881609.
11. Brownlee, M. *Nature*, **2001**, *414*, 813-820. https://doi.org/10.1038/414813a.
12. Jin, H. K.; Nishimura, C. *Diabetes/Metab. Rev.* **1988**, *4*, 323-337. https://doi.org/10.1002/dmr.5610040403.
13. El-Kabbani, O.; Darmanin, C.; Schneider, T. R.; Hazemann, I.; Ruiz, F. M.; Oka, M.; Joachimiak, A.; Schulze-Briese, C.; Tomizaki, T.; Mitschler, A.; Podjarny, A. *Proteins*, **2004**, *55*, 805-813. https://doi.org/10.1002/prot.20001.
14. Nishikawa, T.; Edelstein, D.; Brownlee, M. *Kidney Int.* **2000**, *58*, S26-S30. https://doi.org/10.1046/j.1523-1755.2000.07705.x.
15. Vincent, A. M.; Russell, J. W.; Low, P.; Feldman, E. L. *Endocr. Rev.* **2004**, *25*, 612-628. https://doi.org/10.1210/er.2003-0019.
16. Kador, P. K.; Kinoshita, J. H.; Sharpless, N. E. *J. Med. Chem.* **1985**, *28*, 841-849. https://doi.org/10.1021/jm00145a001.
17. Oates, P. J.; Mylari, B. L. *Expert Opin. Inv. Drug.* **1999**, *8*, 2095–2119. https://doi.org/10.1517/13543784.8.12.2095.
18. Ramasamy, R. *Curr. Drug Targets.* **2003**, *4*, 625–632. https://doi.org/10.2174/1389450033490768.
19. Dvornik, D.; Simard-Duquesne, N.; Krami, M.; Sestanj, K.; Gabbay, K. H.; Kinoshita, J. H.; Varma, S.; Merola, L. O. *Science,* **1973,** *182*, 1146-1148. https://doi.org/10.1126/science.182.4117.1146
20. Terashima, H.; Hama, K.; Yamamoto, R.; Tsuboshima, M.; Kikkawa, R.; Hatanaka, I.; Shigeta, Y. *J. Pharmacol. Exp. Ther.* **1984**, *229*, 226. https://doi.org/10.1016/0160-5402(84)90048-2
21. Sestanj, K.; Bellini, F.; Fung, S.; Abraham, N.; Treasurywala, A.; Humber, L.; Simard-Duquesne, N.; Dvornik, D. *J. Med. Chem.* **1984**, *27*, 255–256. https://doi.org/10.1002/chin.198432157.
22. Mylari, B. L.; Larson, E. R.; Beyer, T. A.; Zembrowski, W. J.; Aldinger, C. E.; Dee, M. F.; Siegel, T.; Singleton, D. H. *J. Med. Chem.* **1991**, *34*, 108-122. https://doi.org/10.1002/chin.199122162
23. Asano, T.; Saito, Y.; Kawakami, M.; Yamada, N. *J. Diabetes Complicat.* **2002**, *16*, 133–138. https://doi.org/10.1016/s1056-8727(01)00175-1
24. Van-Zandt, M. C.; Jones, M. L.; Gunn, D. E.; Geraci, L. S.; Jones, J. H.; Sawicki, D. R.; Sredy, J.; Jacot, J.; Dicioccio, A.; Petrova, T.; Mitschle, A.; Podjarny, A. *J. Med. Chem.* **2005**, *48*, 3141–3152. https://doi.org/10.1021/jm0492094
25. Pitts, N. E.; Vreeland, F.; Shaw, G. L.; Peterson, M. J.; Mehta, D. J.; Collier, J.; Gundersen, K. *Metabolism* **1986**, *35*, 96-100. https://doi.org/10.1016/0026-0495(86)90195-2
26. Hashimoto, K.; Murakami, N.; Ohta, M. Kato, K.; Mizota, M.; Miwa, I.; Okuda, J. *Biol. Pharm. Bul.* **1996**, *19*, 809-813. https://doi.org/10.1248/bpb.19.809
27. Kato, K.; Nakayama, K.; Mizota, M.; Miwa, I.; Okuda, J. *Chem. Pharm. Bull.* **1991**, *39*, 1540-1545. https://doi.org/10.1248/cpb.39.1540
28. Coutcher, J. B.; Dina, M. S.; O'Gorman, M. T.; Linnares, M. C.; Martin, W. H.; Oates, P. J.; Tess, D. A.; Withbroe, G. J.; Zembrowski, W. J. *J. Med. Chem.* **2003**, *46*, 2283–2286. https://doi.org/10.1021/jm034065z
29. Steele, J.W.; Faulds, D.; Goa, K. L. Epalrestat. *Drug. Aging* **1993**, *3*, 532-555. https://doi.org/10.2165/00002512-199303060-00007
30. Kikkawa, R.; Hatanaka, I.; Yasuda, H.; Kobayashi, N.; Shigeta, Y.; Terashima, H.; Morimura, T.; Tsuboshima, M. *Diabetologia* **1983**, *24*, 290-292. https://doi.org/10.1007/BF00282716
31. Bohren, K. M.; Grimshaw, C. E.; Gabbay, K. H. *J. Biol. Chem.* **1992**, *267*, 20965–20970. https://doi.org/10.1111/j.1432-1033.1992.tb17349.x
32. El-Kabbani, O.; Podjarny, A. *Cell. Mol. Life Sci.* **2007**, *64*, 1970-1978. https://doi.org/10.1007/s00018-007-6514-3
33. Feather, M. S.; Flynn, T. G.; Munro, K. A.; Kubiseski, T. J.; Walton, D. J. *Biochim. Biophys. Acta.* **1995**, *1244*, 10–16. https://doi.org/10.1016/0304-4165(94)00156-R
34. Barski, O. A.; Gabbay, K. H.; Bohren, K. M. *Biochemistry*, **1996**, *35*, 14276–14280. https://doi.org/10.1021/bi9619740
35. Urzhumtsev, A.; TêteFavier, F.; Mitschler, A. A; Kubiseski, T. J.; Walton, D. J. *Structure*, **1997**, *5*, 601. https://doi.org/10.1016/S0969-2126(97)00216-5
36. Dudley, D. A.; Bunker, A. M.; Chi, L. L.; Cody, W. L.; Holland, D. R.; Ignasiak, D. P.; Janiczek-Dolphin, N.; McClanahan, T. B.; Mertz, T. E.; Narasimhan, L. S.; Rapundalo, S. T.; Trautschold, J. A.; Van-Huis, C. A.; Edmunds, J. J. *J. Med. Chem.* **2000**, *43*, 4063-4070. https://doi.org/10.1021/jm000074l
37. Brogi, S.; Ramunno, A.; Savi, L.; Chemi, G.; Alfano, G.; Pecorelli, A.; Pambianchi, E.; Galatello, P.; Compagnoni, G.; Focher, F.; Biamonti, G.; Valacchi, G.; Butini, S.; Gemma, S.; Campiani, G.; Brindisi, M. *Eur. J. Med. Chem.* **2017**, *138*, 438-457. http://doi.org/10.1016/j.ejmech.2017.06.017
38. Largerona, M.; Mesples, B.; Gressens, P.; Cecchelli, R.; Spedding, M.; Ridant, A. L.; Fleury, M. B. *Eur. J. Pharmacol.* **2001**, *424*, 189-194. http://doi.org/10.1016/S0014-2999(01)01152-9
39. Tawada, H.; Sugiyama, Y.; Ikeda, H.; Yamamoto, Y.; Meguro, K. *Chem. Pharm. Bull.* **1990**, *38*, 1238-1245. http://doi.org/10.1002/chin.199101247
40. Ohkawa, H.; Ohishi, N.; Yagi, K. *Anal. Biochem.* **1979**, *95*, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3
41. Liu, L.; Liu, Y.; Cui, J.; Liu, Y. B.; Qiao, W. L.; Sun, H.; Yan, C. D. *World J. Gastroenterol.* **2013**, *19*, 9439–9446. https://doi.org/10.3748/wjg.v19.i48.9439
42. El-Kabbani, O.; Carbone, V.; Darmanin, C.; Oka, M.; Mitschler, A.; Podjarny, A.; Schulze-Briese, C.; Chung, R. P. *J. Med. Chem.* **2005**, *48*, 5536-5542. https://doi.org/10.1021/jm050412o
43. Noh, H.; Hu, Y.; Park, T.; Dicioccio, T.; Nichols, A.; Okajima, K.; Homma, S.; Goldberg, I. *J. Pharmacol. Exp. Ther.* **2009**, *328*, 496-503. https://doi.org/10.1124/jpet.108.136283
44. Ye, Q.; Hyndman, D.; Green, N.; Li, L.; Jia, Z.; Flynn, T. *Chem. Biol. Interact.* **2001**, *132*, 651-658. https://doi.org/10.1016/S0009-2797(00)00256-8
45. Qin, X. Y.; Hao, X.; Han, H.; Zhu, S. J.; Yang, Y. C.; Wu, B. B.; Hussain, S.; Parveen, S.; Jing, C. J.; Ma, B.; Zhu, C. J. *J. Med. Chem.* **2015**, *58*, 1254–1267. https://doi.org/10.1021/jm501484b

Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.

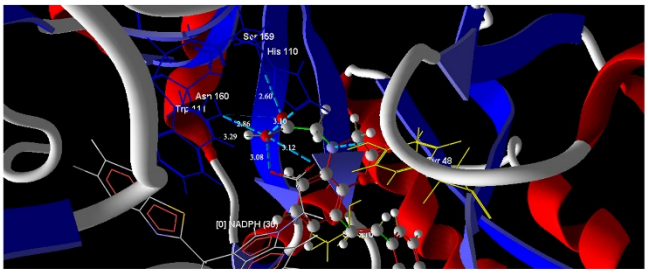
Click here to remove instruction text...

Aldose reductase inhibitors:



dihydrobenzoxazinone derivatives

IC_{50} = 82 nM
good selectiveness
excellent antioxidant



Dihydrobenzoxazinone derivatives revealed potent activities of aldose reductase inhibition and antioxidant against diabetic complications.

√ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: