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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Isatin derivatives as a new class of aldose reductase inhibitors with antioxidant activity

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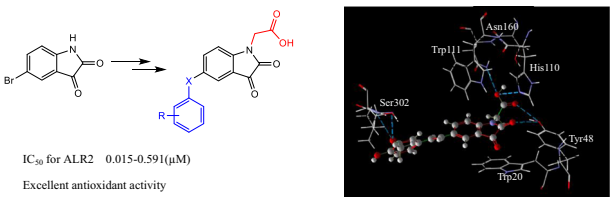
Abstract

In this work, isatin was employed as the scaffold to design aldose reductase inhibitors with antioxidant activity. Most of the isatin derivatives were proved to be excellent in the inhibition of aldose reductase (ALR2) with IC_{50} values at submicromolar level, and (*E*)-2-(5-(4-methoxystyryl)-2,3-dioxoindolin-1-yl) acetic acid (**9g**) was identified as the most effective with an IC_{50} value of 0.015 μM. Moreover, compounds **9a–h** with styryl side chains at the C5 position of isatin showed potent antioxidant activity. Particularly, the phenolic compound **9h** demonstrated similar antioxidant activity with the well-known antioxidant Trolox. Structure-activity relationship and molecular docking studies showed that the acetic acid group at N1 and C5 p-hydroxystyryl side chain were the key structures to increase the aldose reductase inhibitory activity and antioxidant activity.

Graphical Abstract



Keywords Aldose reductase inhibitors · Isatin derivatives · Antioxidant activity · Diabetic complications

Introduction

Diabetes mellitus (DM) is a complex metabolic disease characterized by chronic hyperglycemia caused by absolute or relative deficiency of insulin [1]. Patients with insulin-dependent and noninsulin-dependent DM, are vulnerable to chronic complications such as neuropathy, retinopathy, angiopathy, cataract, nephropathy [2]. Currently, the abnormally activation of glucose metabolism through polyol pathway and increased oxidative stress are thought to be important causes of diabetic complications [3].

As a member of the aldo-keto reductase superfamily, Aldose reductase (ALR2, EC 1.1.1.21) is the first and rate-determining enzyme of the polyol pathway (Fig. 1). It catalyzes the NADPH-dependent reduction of various carbonyl compounds, including glucose [1]. Under normal circumstance, glucose is preferentially phosphorylated with ATP by hexokinase and then enters the glycolytic pathway. Due to the low substrate affinity of ALR2 for glucose, only a small amount of glucose enters the polyol pathway [4]. In the polyol pathway, glucose is reduced to sorbitol which is then oxidized to fructose by sorbitol dehydrogenase (SDH) [5, 6]. Under hyperglycemic state, hexokinase is rapidly saturated and the polyol pathway is activated [7]. ALR2 converts the excess of intracellular glucose to sorbitol which is formed more rapidly than it is converted to fructose. The

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strong polarity of sorbitol makes it difficult to penetrate through membranes and subsequent removal from tissues by diffusion [2]. The abnormal accumulation of sorbitol may cause osmotic imbalance, membrane permeability changes and cell swelling and further develops into diabetic complications [8]. Studies have shown that aldose reductase inhibitors (ARIs) could prevent or slow the progression of DM-associated pathologies both in animal models and humans, which provides a promising strategy for the treatment of diabetic complications [9, 10].

In addition to osmotic imbalance, increased oxidative stress is thought to be another main cause of diabetic complications [11]. There are multiple mechanisms that result in the induction of oxidative stress in DM [12]. Previous studies showed that abnormal activation of the polyol pathway was an important cause of oxidative stress [13–15].

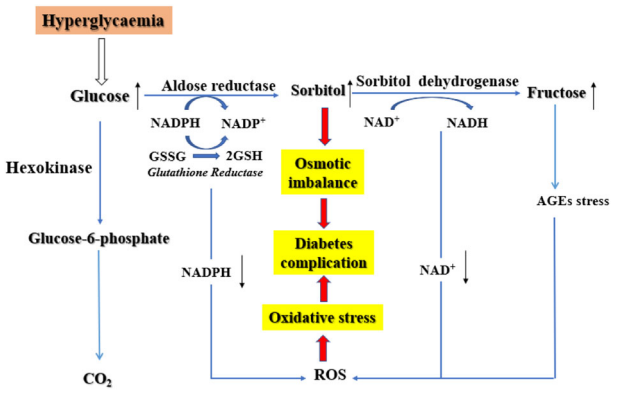


Fig. 1 The polyol pathway of glucose metabolism and related pathogenesis of diabetic complications

Under hyperglycemic circumstance, above 30% of the glucose enters into aldose reductase-dependent polyol pathway, which consumes NADPH and consequently reduces GSH (intracellular antioxidant) level [13, 14]. Moreover, oxidative stress occurs during the conversion of sorbitol into fructose by SDH while the cofactor NAD^+ is converted to NADH [13, 15]. The sharp decrease of NADPH and NAD^+ causes changes of the redox potential in cells, which may lead to the increase of intracellular reactive oxygen species (ROS), ultimately resulting in cellular oxidative stress [16]. Additionally, fructose is metabolized into fructose-3-phosphate and 3-deoxyglucosone which are more potent non-enzymatic glycation agents [13]. These agents increase advanced glycation end products (AGEs) formation, eventually leading to ROS generation and oxidative stress [17]. Thus, ARIs can indirectly inhibit oxidative stress while constraining the intracellular accumulation of sorbitol [18]. Based on this, combining direct antioxidant activity with ALR2 inhibition in the design of new multifuctional ARIs maybe contribute to improve the efficacy of treatment.

During the past few decades, a great deal of ARIs have been reported, and some classical structure of them were shown in Fig. 2 [4]. According to functional groups, they were divided into three categories: sulfonyl derivatives, spiroimide derivatives, and carboxylic acid derivatives [4, 5, 19–21]. As the most classic sulfonyl ARIs, ARI-809 exhibited outstanding inhibitory activity ($IC_{50} < 1$ nM). However, the clinical manifestations of ARI-809 are still unknown [4, 20]. Sorbinil and fidarestat were successful in the inhibition of ALR2 but gave rise to hepatotoxicity and hypersensitivity [4, 19]. Compared with other inhibitors,

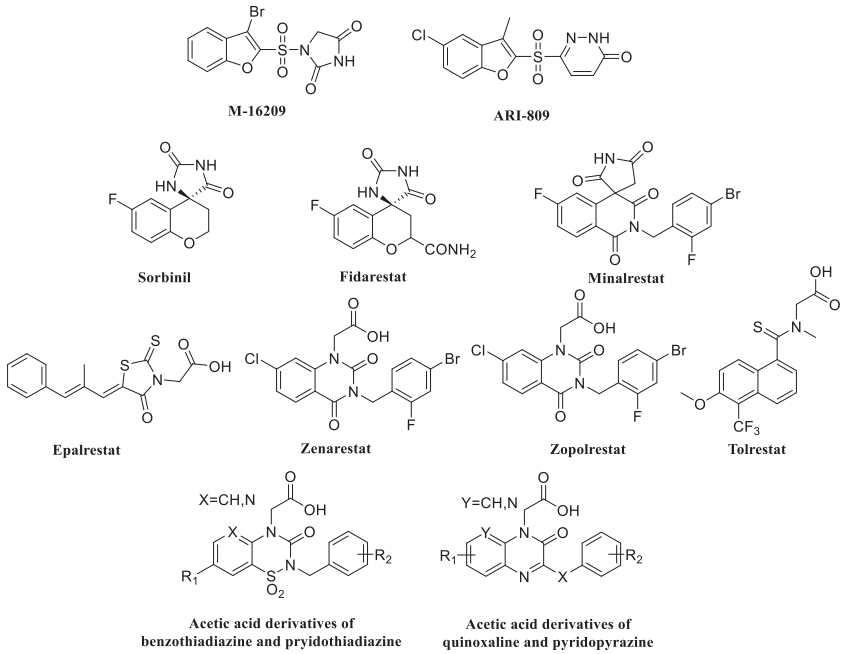
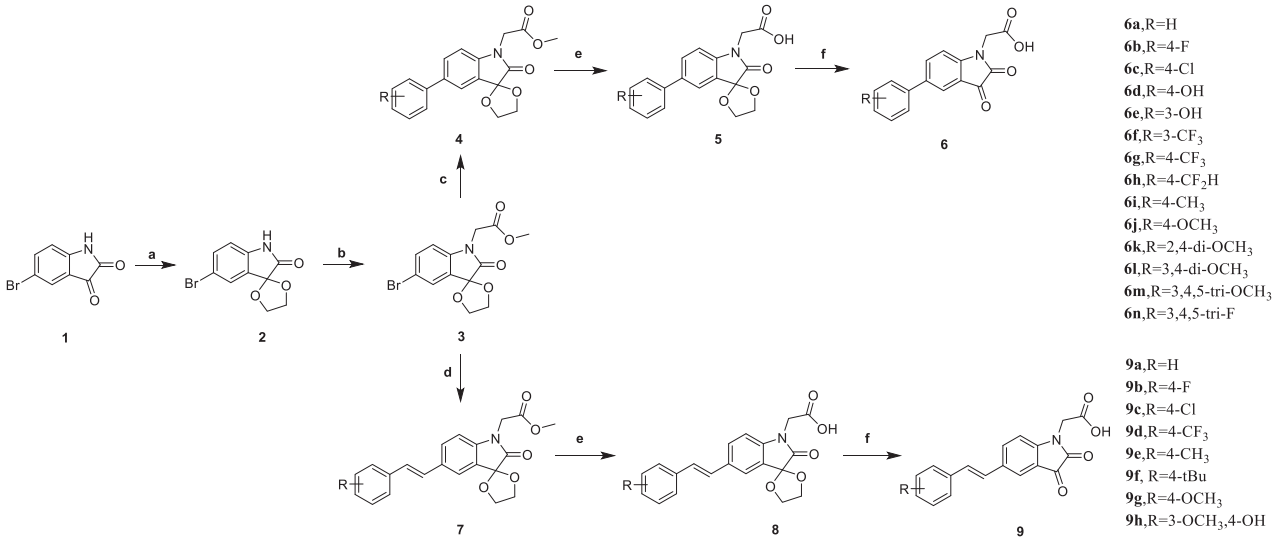


Fig. 2 Classical aldose reductase inhibitor (ARIs) in recent years



Scheme 1 Reagents and conditions: **a** ethylene glycol, p-toluenesulphonic acid, toluene, 130 °C, 3 h, 99%; **b** $BrCH_2CO_2CH_3$, K_2CO_3, CH_3CN, 65 °C, 4 h, 80–88%; **c** $C_6H_5B(OH)_2$, Na_2CO_3, $PdCl_2$ (dppf), 1,4-dioxane, H_2O, 100 °C, 75–85%; **d** styrene, $Pd(OAc)_2$, P(o-tolyl)$_3$, Et_3N, DMF, 100 °C, 50–65%; **e** LiOH, H_2O, THF, rt, 2 h, then 0.1 N HCl, 70–85%; **f** conc HCl, THF, 60 °C, 65–75%

carboxylic acid derivatives serve as potent ARIs in which epalrestat is the only ARI in therapy [4, 21, 22]. It has been marketed in Japan, India, and China. However, some ARIs were limited in their activity, clinical efficacy, and some of them caused unwanted side effects [5]. The crucial reason for the side effects is the lack of selectivity relative to aldehyde reductase (ALR1, EC 1.1.1.2) which catalyzes the metabolism of some toxic aldehydes [23]. ALR1 and ALR2 belong to the same superfamily and have a high degree of homology in many aspects [24]. Ideal ARIs are expected to have little impact on ALR1 while inhibiting ALR2 effectively. Thus, the selectivity for ALR2 must be considered in the design of new ARIs.

Our research group have been committed to the development of new ARIs for many years [1, 8, 16, 23]. Several groups of carboxylic acid ARIs based on structure of pyridothiadiazine, quinoxaline, benzothiadiazine, and pyridopyrazine were developed in recent years (Fig. 2) [16]. In these ARIs, the inhibition of aldose reductase and antioxidation were combined together [1]. Actually, oxidative stress and pathogenesis of the chronic diabetes complications are always interrelated [8]. Inhibiting the activity of ALR2 while reducing the progression of oxidative stress may be a new and effective therapeutic strategy [8]. Isatin (1H-indole-2,3-dione) is ubiquitous in nature, and its derivatives also possess a variety of pharmacological activity such as anticancer, antimalarial, and antioxidant, etc [25–27]. In addition, phenolic hydroxyl groups were widely used in drug design to enhance antioxidant activity [28]. Here, two series of isatin derivatives were synthesized to validate their inhibitory activity, selectivity, and antioxidant effect.

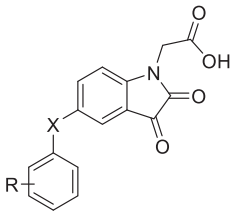
Results and discussion

As shown in Scheme 1, all required compounds were synthesized with 5-bromoisatin as the starting material. Since the Suzuki reaction on 5-bromoisatin is hard to achieve, the free carbonyl (ketone) group may be the key to solving the problem [29, 30]. Thus 5-bromoisatin 1 was converted to the ketal **2** [31]. To afford the key intermediate esters **3**, compound **2** was alkylated at the N1 position with methyl bromoacetate. Different phenyl groups were directly attached to the C5 position of the compound **3** by Suzuki-Miyaura reaction to provide compounds **4**. Then, the desired carboxylic acids **5** were obtained by hydrolysis of **4** with lithium hydroxide. Compounds **5** were heated under reflux of hydrochloric acid to obtain isatin derivatives **6a–n** possessing different substituents. To increase the space length of C5-side chain, styrene derivatives were coupled with **3** through a Heck cross-coupling reaction to produce compounds **7**. The desired compounds **9** were prepared from **7** by the same method of hydrolysis and deprotection.

All of the prepared compounds were assessed for their potential to inhibit ALR2 extracted from rat lenses. In addition, the inhibitory activity against ALR1 isolated from rat kidneys was also tested to evaluate the selectivity of compounds for ALR2 inhibition.

All synthesized compounds were divided into two series according to the different side chains at C5 position of the isatin core. The first series of compounds contained phenyl residues (**6a–n**), while the second series contained styryl residues (**9a–h**). As shown in Table 1, most of the compounds significantly inhibited ALR2, and some of them (**6c**, **6j**, **6m**, and **9a–h**) were even more potent than the positive control

Table 1 Enzyme inhibition activity of isatin derivatives



Comp.	Substit.		IC_{50} for ALR2[a] (μM)	Inhib for ALR1[b] (%)
	R	X		
6a	H	–	0.591 ± 0.054	25.8
6b	4-F	–	0.229 ± 0.081	26.0
6c	4-Cl	–	0.078 ± 0.027	24.9
6d	4-OH	–	0.106 ± 0.005	25.1
6e	3-OH	–	0.184 ± 0.051	23.8
6f	3-CF_3	–	0.160 ± 0.047	35.1
6g	4-CF_3	–	0.133 ± 0.037	23.8
6h	4-CF_2H	–	0.257 ± 0.192	9.9
6i	4-CH_3	–	0.187 ± 0.081	23.8
6j	4-OCH_3	–	0.082 ± 0.024	12.3
6k	2,4-di-OCH_3	–	0.131 ± 0.038	16.2
6l	3,4-di-OCH_3	–	0.158 ± 0.033	19.1
6m	3,4,5-tri-OCH_3	–	0.064 ± 0.015	11.78
6n	3,4,5-tri-F	–	0.156 ± 0.016	<5
9a	H	CH = CH	0.075 ± 0.010	34.4
9b	4-F	CH = CH	0.025 ± 0.009	33.0
9c	4-Cl	CH = CH	0.044 ± 0.018	37.5
9d	4-CF_3	CH = CH	0.029 ± 0.013	29.4
9e	4-CH_3	CH = CH	0.043 ± 0.002	28.8
9f	4-tBu	CH = CH	0.072 ± 0.020	31.25
9g	4-OCH_3	CH = CH	0.015 ± 0.002	36.7
9h	3-OCH_3,4-OH	CH = CH	0.033 ± 0.004	31.9
Epalrestat			0.093 ± 0.006	79.8

[a] $n = 3$

[b]% Inhibition observed at 10 μM

epalrestat. Compounds in the second series with C5-styryl side chain exhibited excellent inhibitory activity against ALR2 with IC_{50} values varying from 0.015 to 0.075 μM, and they were stronger in efficacy than the first series with IC_{50} values ranging from 0.064 to 0.591 μM. Besides, it was found that the vinyl segment between the phenyl and C5 positions of isatin had a positive effect on the improvement of inhibitory activity through the individual comparisons of the two series compounds. For example, **9a**, **9b**, **9c**, and **9g** were more active than their counterparts **6a**, **6b**, **6c**, and **6j**, respectively. Furthermore, all of the alkyl, methoxy, hydroxyl, and halogen substituents at the side of aromatic residue increased the inhibitory activity of the compounds (**6b–n** > **6a**, **9b–h** > **9a**), which resulted from the changes of the electron cloud density on C5 aromatic rings. Therefore, the effect of compounds to inhibit ALR2 was significantly improved by introducing electron-withdrawing or electron-donating groups on the aromatic ring of the C5-side chain. In particular, methoxy

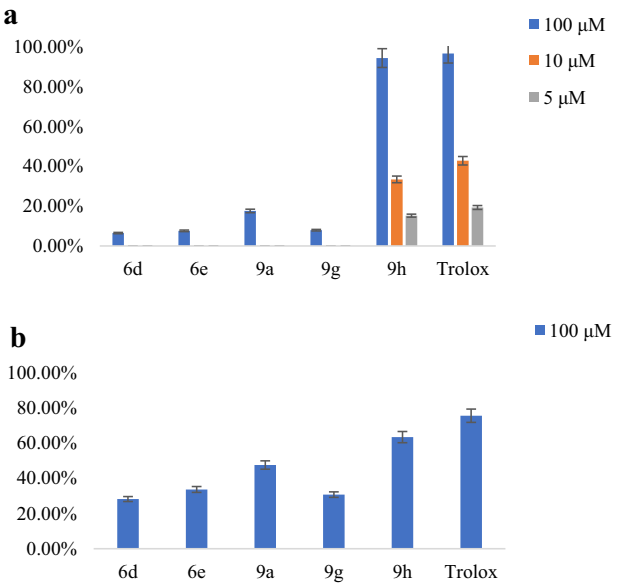


Fig. 3 **a** DPPH radical scavenging activity. **b** Inhibition of lipid peroxidation

group played an important role in ALR2 inhibition. Compounds (**6j–m, 9g–h**) possessing methoxy group demonstrated significant inhibitory activity and **9g** was identified as the best inhibitor with an IC_{50} value of 0.015 μM. By comparing compounds **6d** with **6e** and **6g** with **6f**, it can be concluded that compounds with para-substituents on the side chain had better enzyme inhibitory activity than those with meta-substituents.

All compounds were tested for their inhibitory ability against ALR1 at the concentration of 10 μM. As shown in Table 1, the inhibition percentage of all compounds was no more than 37.5%, which indicated slight effect on ALR1 and good selectivity for ALR2.

Oxidative stress plays an important role in the development of diabetic complications, and the antioxidant activity of compounds having a phenolic hydroxyl group on the side chain was investigated in the present study. Trolox was employed as a positive control. The results of antioxidant test were shown in Fig. 3.

The model reaction with the stable free radical of 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) was applied to measure the free radical scavenging capacity of compounds. Compounds (**9a**, **9g**, and **9h**) containing vinyl spacer exhibited potential antioxidant activity with DPPH radical scavenging rates of 17.6, 7.9, and 94.4% at the concentration of 100 μM. Notably, **9h** showed obvious DPPH radical scavenging activity similar to that of trolox. However, the scavenging effect of **6d** and **6e** on free radicals was insignificant. It showed that the vinyl spacer of the C5-side chain was essential for the DPPH radical scavenging, and it was consistent with the previous study [23]. It should be noted that **9h** was more effective than **9a** and **9g**, indicating that

phenolic hydroxyl group played a key role in improving the DPPH radical scavenging activity. The position of the phenolic hydroxyl group had little effect on the antioxidant activity, which was deduced by the comparison of **6d** and **6e**.

To further evaluate the antioxidant activity of the tested compounds, the experiments of lipid peroxidation suppression were also implemented. As an indicator of lipid peroxidation, thiobarbituric acid reactive materials (TBARs) were produced by the reaction of thiobarbituric acid (TBA) with lipoperoxidation product malondialdehyde (MDA). The results of inhibiting lipid peroxidation test were consistent with the antioxidant results of the DPPH free radical scavenging test (Fig. 3b). Expectedly, compound **9h** performed well-reduced production of TBARs as shown in Fig. 3b. The lipid peroxidation inhibition rate of **9h** was close to that of trolox. As a consequence, compound **9h** exhibited excellent activity in both enzyme inhibition and antioxidant tests, which may provide excellent inspiration for the development of lead structures for ARIs candidates.

According to the study of X-ray crystallography for ALR2, the ALR2 binding site was divided into three subregions: anion binding pocket, specificity pocket, and hydrophobic pocket [32]. Particularly, the specificity pocket is unique to ALR2 and is markedly different from the corresponding pocket of ALR1. Therefore, the specificity pocket is critical to increase the selectivity of compounds for ALR2 inhibition. Based on the above information, molecular docking studies were carried out by software Molegro Virtual Docker, version 5.0.

In order to study the interaction mode of compound with ALR2, the docking of compound **9h** possessing the best activity both in the ALR2 inhibition and antioxidantion was performed with the human ALR2-$NADP^+$-tolmetin complex (PDB code: 3S3G). As shown in Fig. 4a, compound **9h** entered the cavity of ALR2 and bound tightly to the active site. It tucked the carboxyl group into the anion binding pocket and formed strong hydrogen bonds with Tyr48 (3.24 Å), His110 (3.07 Å), Trp111 (3.29 Å), and Asn 160 (3.06 Å) (Fig. 4b).

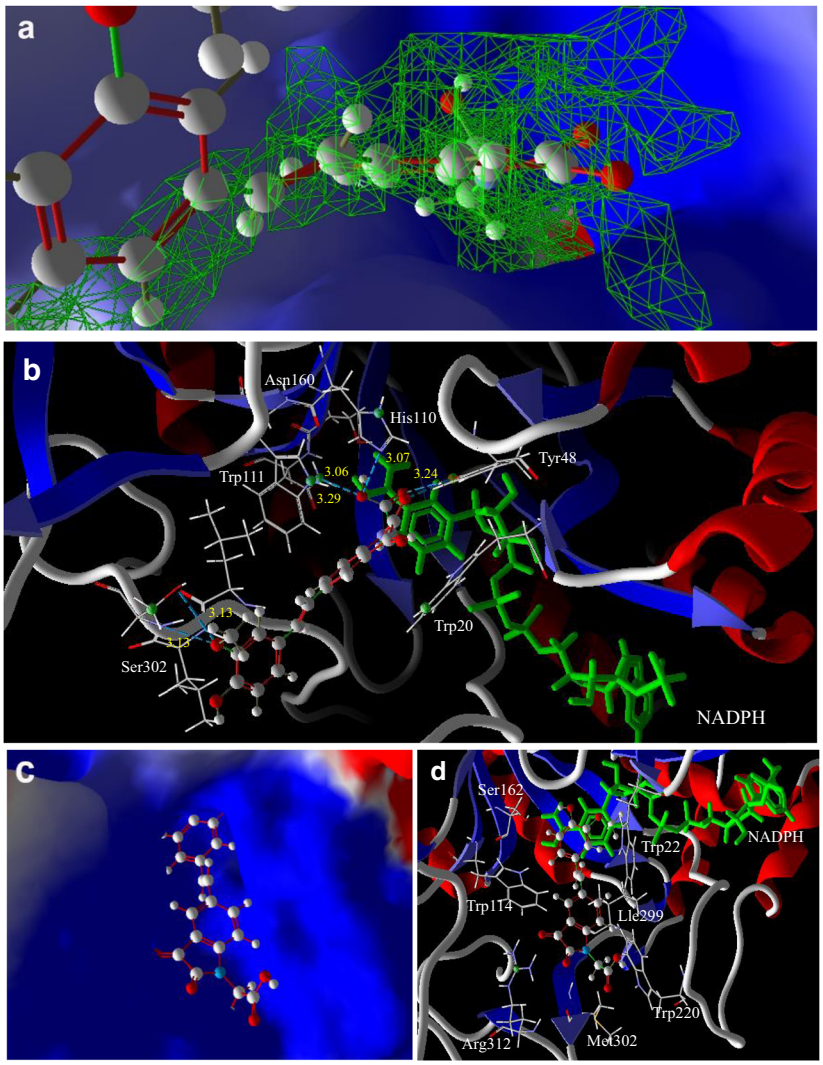


Fig. 4 Molecular docking results of compound **9h** with ALR2 and ALR1. **a**, **c** Surface rendering of protein residues. **b**, **d** The protein structure was represented by ribbons and tubes; the marked specific amino acid residues were shown in lines. The docking posture of **9h** was displayed in white (C, H), red (O), and blue (N). NADPH was displayed in green. The blue dashed line showed the hydrogen bond

This indicated that the acetic acid group at N1 position of isatin played a key role in inhibiting ALR2. Besides, the free carbonyl (ketone) group of isatin core formed an additional hydrogen bond with the side chain of Tyr48 (3.09 Å). Therefore, the free carbonyl (ketone) group of isatin core may be contributed to the improvement of enzyme inhibitory activity. In addition, the 3-methoxy oxygen atom of C5-styryl ring was well embedded into the specificity pocket through two hydrogen bonds with Ser302 (3.13 Å, 3.13 Å), which explains its enhanced ALR2 inhibition. The C5-styryl ring was well placed into the specificity pocket, while the isatin scaffold matched very well with the hydrophobic pocket formed by the side chains of Trp219, Cys298, Trp79, Trp 20, and so on. Therefore, it is obvious that compound **9h** was tightly fixed in the active site of ALR2 by interactions discussed above.

The docking behavior of **9h** with ALR1-$NADP^+$-fidarestat complex (PDB code: 3H4G) was also studied to illustrate its selectivity towards ALR2 over ALR1. The results showed that **9h** did not match with ALR1. The carboxyl group at the N1 position of isatin totally "flowed out" of ALR1 active site, and the compound was almost separated from the surface of ALR1 (Fig. 4c). There was no significant interaction between **9h** and the residues of the active site (Fig. 4d). Maybe that was the reason why compound **9h** exhibited weak effect on ALR1.

Conclusions

In this work, two series of isatin carboxylic acid derivatives were designed as multifunctional ARIs and evaluated for their inhibitory activity and selectivity to ALR2 and antioxidant effects. Most compounds showed remarkable aldose reductase inhibition efficacy and marked selectivity, and **9g** was considered as the most effective with an IC_{50} value of 0.015 μM. Particularly, compound **9h** containing 3-methoxy,4-hydroxyl group at the C5-styryl side chain exhibited the best activity in antioxidant test, which was comparable to that of trolox. The structure-activity relationship showed that the substitution group of C5-styryl side chain were conducive to improving the activity of the compound. In addition, phenolic hydroxyl group was installed in the side chain to reinforce the antioxidant property greatly. In conclusion, compound **9h** containing C5 p-hydroxystyryl side chain demonstrated the best biological activity, which is expected to become a lead compound for multifunctional ARIs.

Experimental section

Chemistry

TLC technique on silica gel Merck 60F254 was used to routinely check all reactions. Melting points of compounds were tested by the X-4 microscopic melting point apparatus. The hydrogen spectrum (^{1}H NMR: 400 MHz) and carbon spectrum (^{13}C NMR: 100 MHz) were obtained by Bruker Ascend 400 MHz nuclear magnetic resonance instrument. Deuterated reagents are $CDCl_3$ and DMSO-d_6, with TMS as internal standard. An AGILENT LC/MS mass spectrometer was used for the determination of HRMS (ESI). The Hitachi D-2000 Elite HPLC system was used for analysis of sample purity. The HPLC conditions are as follows: room temperature; Inertsil ODS-2 250 × 10 mm, 5 mm column; mobile phase: CH_3CN (0.1% TFA)/CH_3OH = 75/25; flow rate is 1 mL/min, last for 8 min. The purity of all compounds used for biological assays was ≥95%.

General procedure for synthesis of 5′-bromospiro[[1,3] dioxolane-2,3′-indolin] -2′-one (2)

General procedure A: 5-bromoisatin (4 g, 17.68 mmol), ethylene glycol (19.6 mL, 338 mmol) and p-toluenesulfonic acid (152.4 mg, 0.88 mmol) were dissolved in solution of toluene (50 mL). The reaction mixture was refluxed at 130 °C for 3 h and then evaporated to dryness. The residue was diluted with DCM and washed with saturated sodium bicarbonate solution. The aqueous layer was extracted three times with DCM. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and evaporated. The crude mixture was purified using column chromatography to give **2** as white powder (4.68 g, 99%).

General procedure for synthesis of methyl-2-(5′-bromo-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (3)

General procedure B: Methyl bromoacetate (1.6 g, 10.5 mmol) was added to a solution of 5′-bromospiro[[1,3] dioxolane-2,3′-indolin] -2′-one (**2**) (10 mmol) in K_2CO_3 (4.15 g, 30 mmol) and CH_3CN (50 mL). The reaction mixture was heated to 65 °C over 2 h, then filtered, concentrated in vacuo and recrystallized to give **3** as white powder (3.2 g, 95%).

General procedure for synthesis of methyl-2-(5′-phenyl-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate derivatives (4a–4n)

General procedure C: A mixture of methyl-2-(5′-bromo-2′-oxospiro[[1,3] dioxolane-2,3′-indoline]-1′-yl) acetate **3** (1 mmol), phenylboronic acid (1.1 mmol), Na_2CO_3 (2 mmol), $PdCl_2$(dppf) (0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) was stirred at 100 °C under argon for 8 h. After the reaction was completed, the reaction mixture was washed with brine (2 × 200 mL), extracted with 40 mL of EtOAc three times, dried ($MgSO_4$) and concentrated in vacuo. The crude mixture was purified by silica gel chromatography with petroleum ether/ethyl acetate (4:1) to

afford methyl-2-(5′-phenyl-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate derivatives (**4a**–**4n**) with 70–80% yield.

Methyl-2-(5′-phenyl-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4a) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), phenylboronic acid (134.2 mg, 1.1 mmol), Na_2CO_3 (212 mg, 2 mmol), $PdCl_2$(dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4a** as white powder (271 mg, 80%).

Methyl-2-(5′-(4-fluorophenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4b) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (4-fluorophenyl)boronic acid (154 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$(dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4b** as white powder (280 mg, 79%).

Methyl-2-(5′-(4-chlorophenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4c) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (4-chlorophenyl)boronic acid (171.6 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$(dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4c** as white powder (298 mg, 80%).

Methyl-2-(5′-(4-hydroxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4d) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (4-hydroxyphenyl) boronic acid (151.6 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$ (dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4d** as white powder (277 mg, 78%).

Methyl-2-(5′-(3-hydroxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4e) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (3-hydroxyphenyl)boronic acid (151.6 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$(dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4e** as white powder (277 mg, 78%).

Methyl-2-(5′-(3-trifluoromethylphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4f) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (3-trifluoromethylphenyl)boronic acid (209 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$ (dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4f** as white powder (271 mg, 80%).

Methyl-2-(5′-(4-trifluoromethylphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4g) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (4-trifluoromethylphenyl) boronic acid (209 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$(dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4g** as white powder (309 mg, 76%).

Methyl-2-(5′-(4-difluoromethylphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4h) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (4-difluoromethylphenyl) boronic acid (189.2 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$(dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4h** as white powder (299 mg, 77%).

Methyl-2-(5′-(p-tolyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4i) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), p-tolylboronic acid (149.6 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$(dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4i** as white powder (271 mg, 77%).

Methyl-2-(5′-(methoxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4j) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (4-methoxyphenyl)boronic acid (167.2 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$(dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4j** as white powder (287 mg, 78%).

Methyl-2-(5′-(2,4-dimethoxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4k) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (2,4-dimethoxyphenyl) boronic acid (200 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$(dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4k** as white powder (299 mg, 75%).

Methyl-2-(5′-(3,4-dimethoxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4l) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (3,4-dimethoxyphenyl) boronic acid (200 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$(dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4l** as white powder (299 mg, 75%).

Methyl-2-(5′-(3,4,5-trimethoxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4m) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (3,4,5-trimethoxyphenyl)boronic acid (233 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$ (dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4m** as white powder (321 mg, 80%).

Methyl-2-(5′-(3,4,5-trifluorophenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (4n) The reaction was carried out according to the general procedure C using compound **3** (314 mg, 1 mmol), (3,4,5-trifluorophenyl)boronic acid (193.6 mg, 1.1 mmol), Na_2CO_3 (202 mg, 2 mmol), $PdCl_2$ (dppf) (36.5 mg, 0.05 mmol), H_2O (1 mL) and 1,4-dioxane (13 mL) to give **4n** as white powder (298 mg, 76%).

General procedure for synthesis of methyl (E)-2-(5′-styryl-2′-oxospiro-[[1,3] dioxolane -2,3′-indoline]-1′-yl) acetate derivatives (7a–7h)

General procedure D: A mixture of methyl-2-(5′-bromo-2′-oxospiro[[1,3] dioxolane-2,3′-indoline]-1′-yl) acetate **3** (1 mmol), $Pd(OAc)_2$ (6.73 mg, 0.03 mmol), P(o-tolyl)$_3$ (21.31 mg, 0.07 mmol) and DMF(10 mL) was stirred at room temperature under argon for 10 min. Then, styrene (1.5 mmol) and dry Et_3N (0.30 g, 3 mmol) were added. The reaction mixture was heated at 100 °C for 8 h, then washed with brine (2 × 200 mL), extracted with 50 mL of EtOAc three times, dried ($MgSO_4$) and concentrated in vacuo to afford the crude reaction intermediate. Purification by column chromatography afforded methyl (*E*)-2-(5′-styryl-2′-oxospiro-[[1,3] dioxolane -2,3′-indoline]-1′-yl) acetate derivatives with 60–70% yield.

methyl (E)-2-(5′-styryl-2′-oxospiro-[[1,3] dioxolane -2,3′-indoline]-1′-yl) acetate (7a) The reaction was carried out according to the general procedure D using compound **3** (314 mg, 1 mmol), $Pd(OAc)_2$ (6.73 mg, 0.03 mmol), P(o-tolyl)$_3$ (21.31 mg, 0.07 mmol), styrene (156 mg, 1.5 mmol), dry Et_3N (0.30 g, 3 mmol) and DMF(10 mL) to give **7a** as white powder (219 mg, 60%).

methyl (E)-2-(5′-(4-fluorostyryl)-2′-oxospiro-[[1,3] dioxolane -2,3′-indoline]-1′-yl) acetate (7b) The reaction was carried out according to the general procedure D using compound **3** (314 mg, 1 mmol), $Pd(OAc)_2$ (6.73 mg, 0.03 mmol), P(o-tolyl)$_3$ (21.31 mg, 0.07 mmol), 1-fluoro-4-vinylbenzene (183 mg, 1.5 mmol), dry Et_3N (0.30 g, 3 mmol) and DMF (10 mL) to give **7b** as white powder (237 mg, 62%).

methyl (E)-2-(5′-(4-chlorostyryl)-2′-oxospiro-[[1,3]dioxolane -2,3′-indoline]-1′-yl) acetate (7c) The reaction was carried out according to the general procedure D using compound **3** (314 mg, 1 mmol), $Pd(OAc)_2$ (6.73 mg, 0.03 mmol), P(o-tolyl)$_3$ (21.31 mg, 0.07 mmol), 1-chloro-4-vinylbenzene (207 mg, 1.5 mmol), dry Et_3N (0.30 g, 3 mmol) and DMF (10 mL) to give **7c** as white powder (240 mg, 60%).

methyl (E)-2-(5′-(4- trifluoromethylstyryl)-2′-oxospiro-[[1,3] dioxolane-2,3′-indoline]-1′-yl) acetate (7d) The reaction was carried out according to the general procedure D using compound **3** (314 mg, 1 mmol), $Pd(OAc)_2$ (6.73 mg, 0.03 mmol), P(o-tolyl)$_3$ (21.31 mg, 0.07 mmol), 1-(trifluoromethyl)-4-vinylbenzene (258 mg, 1.5 mmol), dry Et_3N (0.30 g, 3 mmol) and DMF(10 mL) to give **7d** as white powder (241 mg, 60%).

methyl (E)-2-(5′-(4-methylstyryl)-2′-oxospiro-[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (7e) The reaction was carried out according to the general procedure D using compound **3** (314 mg, 1 mmol), $Pd(OAc)_2$ (6.73 mg, 0.03 mmol), P(o-tolyl)$_3$ (21.31 mg, 0.07 mmol), 1-methyl-4-vinylbenzene (177 mg, 1.5 mmol), dry Et_3N (0.30 g, 3 mmol) and DMF(10 mL) to give **7e** as white powder (238 mg, 63%).

methyl (E)-2-(5′-(4-tert-butylstyryl)-2′-oxospiro-[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (7f) The reaction was carried out according to the general procedure D using compound **3** (314 mg, 1 mmol), $Pd(OAc)_2$ (6.73 mg, 0.03 mmol), P(o-tolyl)$_3$ (21.31 mg, 0.07 mmol), 1-(tert-butyl)-4-vinylbenzene (240 mg, 1.5 mmol), dry Et_3N (0.30 g, 3 mmol) and DMF (10 mL) to give **7f** as white powder (250 mg, 60%).

methyl (E)-2-(5′-(4-methoxystyryl)-2′-oxospiro-[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (7g) The reaction was carried out according to the general procedure D using compound **3** (314 mg, 1 mmol), $Pd(OAc)_2$ (6.73 mg, 0.03 mmol), P(o-tolyl)$_3$ (21.31 mg, 0.07 mmol), 1-methoxy-4-vinylbenzene (201 mg, 1.5 mmol), dry Et_3N (0.30 g, 3 mmol) and DMF(10 mL) to give **7g** as white powder (248 mg, 63%).

methyl (E)-2-(5′-(4-hydroxy-3-methoxystyryl)-2′-oxospiro-[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate (7h) The reaction was carried out according to the general procedure D using compound **3** (314 mg, 1 mmol), $Pd(OAc)_2$ (6.73 mg, 0.03 mmol), P(o-tolyl)$_3$ (21.31 mg, 0.07 mmol), 2-methoxy-4-vinylphenol (225 mg, 1.5 mmol), dry Et_3N (0.30 g, 3 mmol) and DMF(10 mL) to give **7h** as white powder (250 mg, 63%).

General procedure for synthesis of 2-(5′-phenyl-2′-oxospiro [[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid derivatives (5a–5n)

General hydrolysis procedure E: A mixture of methyl-2-(5′-phenyl-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl)

acetate derivatives (**4**) (0.6 mmol) and saturated aq LiOH (5 mL) in THF (4 mL) was stirred at rt for 2 h. Then, the alkaline suspension was acidified with 0.1 N HCl to pH 3. The resulting precipitate was collected by filtration, washed with H_2O, dried in vacuo and recrystallized from CH_3OH to give desired final product **5** with 70–85% yield.

2-(5′-phenyl-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5a) The reaction was carried out according to the general procedure E using compound **4a** (203 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5a** as white powder (156 mg, 80%).

2-(5′-(4-fluorophenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5b) The reaction was carried out according to the general procedure E using compound **4b** (214 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5b** as white powder (162 mg, 79%).

2-(5′-(4-chlorophenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl)acetic acid (5c) The reaction was carried out according to the general procedure E using compound **4c** (223 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5c** as white powder (168 mg, 78%).

2-(5′-(4-hydroxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5d) The reaction was carried out according to the general procedure E using compound **4d** (223 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5d** as white powder (165 mg, 81%).

2-(5′-(3-hydroxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5e) The reaction was carried out according to the general procedure E using compound **4e** (223 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5e** as white powder (165 mg, 81%).

2-(5′-(3-trifluoromethylphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5f) The reaction was carried out according to the general procedure E using compound **4f** (244.2 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5f** as white powder (190 mg, 81%).

2-(5′-(4-trifluoromethylphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5g) The reaction was carried out according to the general procedure E using compound **4g** (244.2 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5g** as white powder (175 mg, 78%).

2-(5′-(4-difluoromethylphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5h) The reaction was carried out according to the general procedure E using compound **4h** (233.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5h** as white powder (175 mg, 78%).

2-(5′-(p-tolyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5i) The reaction was carried out according to the general procedure E using compound **4i** (211.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5i** as white powder (152 mg, 75%).

2-(5′-(methoxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetate acetic acid (5j) The reaction was carried out according to the general procedure E using compound **4j** (221.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5j** as white powder (161 mg, 76%).

2-(5′-(2,4-dimethoxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5k) The reaction was carried out according to the general procedure E using compound **4k** (239.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5k** as white powder (180 mg, 78%).

2-(5′-(3,4-dimethoxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl)acetic acid (5l) The reaction was carried out according to the general procedure E using compound **4l** (239.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5l** as white powder (180 mg, 78%).

2-(5′-(3,4,5-trimethoxyphenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5m) The reaction was carried out according to the general procedure E using compound **4m** (257.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5m** as white powder (199 mg, 80%).

2-(5′-(3,4,5-trifluorophenyl)-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (5n) The reaction was carried out according to the general procedure E using compound **4n** (235.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **5n** as white powder (172 mg, 76%).

General procedure for synthesis of (*E*)-2-(5′-styryl-2′-oxospiro-[[1,3] dioxolane -2,3′-indoline]-1′-yl) acetic acid derivatives (8a–8h)

Hydrolysis was the same as described above in general hydrolysis procedure E to give desired product **8** with 70–85% yield.

(E)-2-(5′-styryl-2′-oxospiro-[[1,3] dioxolane -2,3′-indoline]-1′-yl) (8a) The reaction was carried out according to the general procedure E using compound **7a** (219.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **8a** as white powder (157 mg, 75%).

(E)-2-(5′-(4-fluorostyryl)-2′-oxospiro-[[1,3] dioxolane -2,3′-indoline]-1′-yl) (8b) The reaction was carried out according to the general procedure E using compound **7b** (229.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **8b** as white powder (170 mg, 77%).

(E)-2-(5′-(4-chlorostyryl)-2′-oxospiro-[[1,3]dioxolane -2,3′-indoline]-1′-yl)acetic acid (8c) The reaction was carried out according to the general procedure E using compound **7c** (239.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **8c** as white powder (166 mg, 72%).

(E)-2-(5′-(4-trifluoromethylstyryl)-2′-oxospiro-[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (8d) The reaction was carried out according to the general procedure E using compound **7d** (259.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **8d** as white powder (178 mg, 71%).

(E)-2-(5′-(4-methylstyryl)-2′-oxospiro-[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (8e) The reaction was carried out according to the general procedure E using compound **7e** (227.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **8e** as white powder (164 mg, 75%).

(E)-2-(5′-(4-tert-butylstyryl)-2′-oxospiro-[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (8f) The reaction was carried out according to the general procedure E using compound **7f** (252.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **8f** as white powder (178 mg, 73%).

(E)-2-(5′-(4-methoxystyryl)-2′-oxospiro-[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid (8g) The reaction was carried out according to the general procedure E using compound **7g** (237.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **8g** as white powder (169 mg, 74%).

(E)-2-(5′-(4-hydroxy-3-methoxystyryl)-2′-oxospiro-[[1,3] dioxolane-2,3′-indoline]-1′-yl) acetic acid (8h) The reaction was carried out according to the general procedure E using compound **7h** (246.4 mg, 0.6 mmol), saturated aq LiOH (5 mL) and THF (4 mL) to give **8h** as white powder (185 mg, 78%).

General procedure for synthesis of 2-(2,3-dioxo-5-phenylindolin-1-yl)acetic acid derivatives (6a–6n)

General procedure F: Concentrated HCl (2 mL) was added to a solution of 2-(5′-phenyl-2′-oxospiro[[1,3]dioxolane-2,3′-indoline]-1′-yl) acetic acid derivatives (**5**) (0.5 mmol) in THF(8 mL) and the mixture was allowed to reflux at 60 °C for 4 h. After the reaction was completed, the acidic suspension was alkalified with Na_2CO_3 to pH 10. Then the impurities were extracted from the water by ethyl acetate and the aqueous phase was collected. For the aqueous phase, the alkaline suspension was acidified with 0.1 N HCl to pH 3. The resulting precipitate was collected by filtration, washed with H_2O, dried in vacuo, and recrystallized to give desired final product **6** with 65–75% yield.

2-(2,3-dioxo-5-phenylindolin-1-yl)acetic acid (6a) The reaction was carried out according to the general procedure F using compound **5a** (162.5 mg, 0.5 mmol), cncentrated HCl (2 mL) and THF (8 mL) to give **6a** as red powder (98 mg, 70%). M.p. 182–184 °C; purity: 99.45%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.31 (s, 1H), 8.01 (dd, $J = 8.3$, 2.0 Hz, 1H), 7.86 (d, $J = 1.9$ Hz, 1H), 7.71–7.68 (m, 2H), 7.47 (t, $J = 7.6$ Hz, 2H), 7.39 (d, $J = 7.3$ Hz, 1H), 7.29 (d, $J = 8.3$ Hz, 1H), 4.53 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.41, 169.37, 158.78, 150.48, 138.96, 137.03, 136.16, 129.50(2 C), 128.13, 126.87(2 C), 122.86, 118.31, 112.17, 42.01 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 280.07, found 280.0624.

2-(5-(4-fluorophenyl)-2,3-dioxoindolin-1-yl)acetic acid (6b) The reaction was carried out according to the general procedure F using compound **5b** (171.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6b** as red powder (103 mg, 69%). M.p. 238–240 °C; purity: 98.76%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.34 (s, 1H), 7.99 (dd, $J = 8.3$, 2.0 Hz, 1H), 7.86 (d, $J = 1.9$ Hz, 1H), 7.77–7.73 (m, 2H), 7.31–7.27 (m, 3H), 4.55 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.31, 169.30, 158.77, 150.36, 136.98, 135.48, 135.18, 128.97 (d, $J = 8.1$ Hz)(2 C), 122.91, 118.30, 116.36, 116.15(2 C), 112.15, 41.89 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 298.06, found 298.0517.

2-(5-(4-chlorophenyl)-2,3-dioxoindolin-1-yl)acetic acid (6c) The reaction was carried out according to the general procedure F using compound **5c** (179.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6c** as red powder (111 mg, 71%). M.p. 214–216 °C; purity: 99.94%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.29 (s, 1H), 8.01 (dd, $J = 8.3$, 2.0 Hz, 1H), 7.88 (d, $J = 1.8$ Hz, 1H), 7.74 (d, $J = 8.5$ Hz, 2H), 7.51 (d, $J = 8.6$ Hz, 2H), 7.32 (d, $J = 8.3$ Hz, 1H), 4.57 (s, 2H). ^{13}C NMR (176 MHz, DMSO-d_6) δ 183.18, 169.31, 158.78, 150.55, 137.77, 136.97, 134.82, 132.99, 129.39, 128.68, 122.95, 118.34, 112.20, 41.82 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 314.03, found 314.0222.

2-(5-(4-hydroxyphenyl)-2,3-dioxoindolin-1-yl)acetic acid (6d) The reaction was carried out according to the general procedure F using compound **5d** (171.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6d** as red powder (108 mg, 73%). M.p. 230–232 °C; purity: 99.39%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.28 (s, 1H), 9.60 (s, 1H),

7.90 (dd, $J = 8.3$, 2.0 Hz, 1H), 7.75 (d, $J = 1.9$ Hz, 1H), 7.51 (d, $J = 8.6$ Hz, 2H), 7.26–7.23 (m, 1H), 6.84 (d, $J = 8.6$ Hz, 2H), 4.54 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.45, 169.34, 158.79, 157.80, 149.53, 136.45, 136.23, 129.70, 128.00 (2C), 122.10, 118.24, 116.26 (2C), 112.02, 41.79 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 296.06, found 296.0566.

2-(5-(3-hydroxyphenyl)-2,3-dioxoindolin-1-yl)acetic acid (6e) The reaction was carried out according to the general procedure F using compound **5e** (171.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6e** as red powder (108 mg, 73%). M.p. 238–240 °C; purity: 99.94%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.22 (s, 1H), 9.57 (s, 1H), 7.94 (dd, $J = 8.3$, 1.9 Hz, 1H), 7.76 (d, $J = 1.7$ Hz, 1H), 7.29–7.24 (m, 2H), 7.09 (d, $J = 7.8$ Hz, 1H), 7.02 (d, $J =$ 1.8 Hz, 1H), 6.79 (dd, $J = 8.0$, 1.8 Hz, 1H), 4.55 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.29, 169.3, 158.79, 158.37, 150.31, 140.37, 136.93, 136.37, 130.55, 122.70, 118.25, 117.62, 115.17, 113.62, 112.10, 41.83 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 296.06, found 296.0575.

2-(2,3-dioxo-5-(3-(trifluoromethyl)phenyl)indolin-1-yl)acetic acid (6f) The reaction was carried out according to the general procedure F using compound **5f** (196.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6f** as red powder (115 mg, 66%). M.p. 172–174 °C; purity: 99.47%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.26 (s, 1H), 8.11 (dd, $J = 8.3$, 2.0 Hz, 1H), 8.02 (dd, $J = 10.9$, 5.4 Hz, 3H), 7.74–7.69 (m, 2H), 7.34 (d, $J = 8.3$ Hz, 1H), 4.57 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.14, 169.30, 158.79, 150.90, 140.03, 137.41, 134.51, 131.01, 130.72–130.14 (2C), 130.17, 126.01, 124.64, 123.48, 118.38, 112.23, 41.87 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 348.06, found 348.0507.

2-(2,3-dioxo-5-(4-(trifluoromethyl)phenyl)indolin-1-yl)acetic acid (6g) The reaction was carried out according to the general procedure F using compound **5g** (196.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6g** as red powder (110 mg, 65%). M.p. 202–204 °C; purity: 99.49%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.30 (s, 1H), 8.10 (dd, $J = 8.3$, 1.9 Hz, 1H), 7.97 (dd, $J = 10.4$, 5.0 Hz, 3H), 7.82 (d, $J = 8.2$ Hz, 2H), 7.36 (d, $J = 8.3$ Hz, 1H), 4.58 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.10, 169.29, 158.79, 151.06, 142.94, 137.40, 134.46, 128.58, 128.26, 127.68 (2C), 126.27 (d, 2C), 123.37, 118.42, 112.29, 41.87 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 348.06, found 348.0496.

2-(5-(4-(difluoromethyl)phenyl)-2,3-dioxoindolin-1-yl)acetic acid (6h) The reaction was carried out according to the general procedure F using compound **5h** (187.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6h** as red powder (112 mg, 68%). M.p. 218–220 °C; purity: 99.83%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.20 (s, 1H), 8.12 (dd, $J = 8.3$, 1.9 Hz, 1H), 7.99 (d, $J = 1.7$ Hz, 1H), 7.91 (d, $J = 8.1$ Hz, 2H), 7.72 (d, $J = 8.1$ Hz, 2H), 7.39 (d, $J =$ 8.3 Hz, 1H), 7.14 (t, $J = 55.9$ Hz, 1H), 4.62 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.19, 169.30, 158.79, 150.79, 141.41, 137.24, 135.10, 127.30(2C), 126.83 (t, $J = 5.9$ Hz), 123.18, 118.39, 117.67, 115.33, 112.98, 112.23, 41.87 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 330.07, found 330.0589.

2-(2,3-dioxo-5-(p-tolyl)indolin-1-yl)acetic acid (6i) The reaction was carried out according to the general procedure F using compound **5i** (169.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6i** as red powder (101 mg, 69%). M.p. 210–212 °C; purity: 99.84%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.26 (s, 1H), 7.98 (dd, $J = 8.3$, 1.9 Hz, 1H), 7.83 (d, $J = 1.8$ Hz, 1H), 7.59 (d, $J = 8.1$ Hz, 2H), 7.28 (dd, $J = 8.1$, 3.2 Hz, 3H), 4.56 (s, 2H), 2.35 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.34, 169.32, 158.79, 150.12, 137.52, 136.73, 136.12 (d, $J = 12.6$ Hz,2C), 130.08 (2C), 126.68 (2C), 122.60, 118.29, 112.10, 41.82, 21.12 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 294.08, found 294.0772.

2-(5-(4-methoxyphenyl)-2,3-dioxoindolin-1-yl)acetic acid (6j) The reaction was carried out according to the general procedure F using compound **5j** (177.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6j** as red powder (105 mg, 68%). M.p. 192–194 °C; purity: 99.74%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.27 (s, 1H), 7.95 (dd, $J = 8.3$, 2.0 Hz, 1H), 7.81 (d, $J = 1.8$ Hz, 1H), 7.65–7.62 (m, 2H), 7.27 (d, $J = 8.3$ Hz, 1H), 7.04–7.00 (m, 2H), 4.55 (s, 2H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.41, 169.33, 159.54, 158.79, 149.80, 136.47, 136.01, 131.31, 128.03(2 C), 122.35, 118.28, 114.91(2C), 112.08, 55.69, 41.81 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 310.08, found 310.0712.

2-(5-(2,4-dimethoxyphenyl)-2,3-dioxoindolin-1-yl)acetic acid (6k) The reaction was carried out according to the general procedure F using compound **5k** (192.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6k** as red powder (114 mg, 67%). M.p. 210–212 °C; purity: 98.18% ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.28 (s, 1H), 7.73 (dd, $J = 8.2$, 1.8 Hz, 1H), 7.61 (d, $J = 1.6$ Hz, 1H), 7.26 (d, $J = 8.4$ Hz, 1H), 7.21 (d, $J = 8.3$ Hz, 1H), 6.68 (d, $J = 2.3$ Hz, 1H), 6.63 (dd, $J = 8.4$, 2.3 Hz, 1H), 4.54 (s, 2H), 3.81 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.43, 169.36, 160.87, 158.81, 157.52, 149.47, 139.41, 133.92, 131.05, 125.32, 121.05, 117.54, 111.34, 105.92, 99.42, 56.10, 55.80, 41.74 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 340.09, found 340.0842.

2-(5-(3,4-dimethoxyphenyl)-2,3-dioxoindolin-1-yl)acetic acid (6l) The reaction was carried out according to the

general procedure F using compound **5l** (192.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6l** as red powder (114 mg, 67%). M.p. 208–210 °C; purity: 99.76%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.27 (s, 1H), 7.99 (dd, $J = 8.3$, 1.9 Hz, 1H), 7.88 (d, $J = 1.7$ Hz, 1H), 7.27 (t, $J = 5.0$ Hz, 2H), 7.23 (dd, $J = 8.3$, 2.0 Hz, 1H), 7.03 (d, $J = 8.4$ Hz, 1H), 4.56 (s, 2H), 3.86 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.44, 169.35, 158.82, 149.86, 149.60, 149.13, 136.69, 136.29, 131.66, 122.68, 119.02, 118.22, 112.63, 111.97, 110.63, 56.07 (d, $J = 4.0$ Hz, 2C), 41.81 ppm. HRMS (ESI) m/z calcd for $[M + H]^+$ 342.09, found 342.0959.

2-(2,3-dioxo-5-(3,4,5-trimethoxyphenyl)indolin-1-yl)acetic acid (6m) The reaction was carried out according to the general procedure F using compound **5m** (207.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6m** as red powder (127 mg, 69%). M.p. 200–202 °C; purity: 99.36%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (dd, $J = 8.3$, 1.7 Hz, 1H), 7.93 (d, $J = 1.5$ Hz, 1H), 7.28 (d, $J = 8.3$ Hz, 1H), 6.94 (s, 2H), 4.56 (s, 2H), 3.87 (s, 6H), 3.69 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 188.15, 174.09, 163.58, 158.50(2 C), 155.00, 142.47, 141.91, 141.14, 139.45, 127.97, 122.92, 116.65, 109.16(2C), 65.27, 61.25 (2C), 46.58 ppm. HRMS (ESI) m/z calcd for $[M + H]^+$ 372.10, found 372.1060.

2-(2,3-dioxo-5-(3,4,5-trifluorophenyl)indolin-1-yl)acetic acid (6n) The reaction was carried out according to the general procedure F using compound **5n** (189.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **6n** as red powder (118 mg, 71%). M.p. 238–240 °C; purity: 99.25%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.29 (s, 1H), 8.07 (dd, $J = 8.4$, 2.0 Hz, 1H), 7.99 (d, $J = 1.9$ Hz, 1H), 7.78 (dd, $J = 9.6$, 6.7 Hz, 2H), 7.33 (d, $J = 8.4$ Hz, 1H), 4.57 (s, 2H). ^{13}C NMR (176 MHz, DMSO-d_6) δ 183.03, 169.29, 158.77, 151.80, 151.06, 150.40, 137.12, 135.70, 132.77, 123.35, 118.30, 112.15(2 C), 111.57 (d, $J = 18.3$ Hz), 41.84 ppm. HRMS (ESI) m/z calcd for $[M + H]^+$ 336.04, found 336.0456.

General procedure for synthesis of (*E*)-2-(2,3-dioxo-5-styrylindolin-1-yl)acetic acid derivatives (9a–9h)

Deprotection was the same as described above in general procedure F to give desired product **9** with 65–75% yield.

(E)-2-(2,3-dioxo-5-styrylindolin-1-yl)acetic acid (9a) The reaction was carried out according to the general procedure F using compound **8a** (175.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **9a** as red powder (101 mg, 66%). M.p. 268–270 °C; purity: 98.30%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.37 (s, 1H), 7.89 (dd, $J = 8.0$, 6.3 Hz, 2H), 7.59 (d, $J = 7.4$ Hz, 2H), 7.37 (d, $J = 7.8$ Hz, 2H), 7.30–7.26 (m, 3H), 7.19 (d, $J = 8.2$ Hz, 1H), 4.46 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.53, 169.37, 158.77, 150.24, 137.38, 136.70, 133.40, 129.19 (2C), 128.81, 128.17, 127.22, 126.94 (2C), 122.38, 118.19, 111.90, 42.12 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 306.08, found 306.0771.

(E)-2-(5-(4-fluorostyryl)-2,3-dioxoindolin-1-yl)acetic acid (9b) The reaction was carried out according to the general procedure F using compound **8b** (184.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **9b** as red powder (110 mg, 68%). M.p. 272–274 °C; purity: 98.50%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.35 (s, 1H), 7.88 (dd, $J = 10.1$, 1.8 Hz, 2H), 7.65–7.61 (m, 2H), 7.32 (d, $J = 16.5$ Hz, 1H), 7.25–7.23 (m, 2H), 7.22–7.20 (m, 2H), 4.52 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.38, 169.29, 160.94, 158.78, 150.06, 136.68, 134.00, 133.41, 128.80 (d, $J = 8.0$ Hz, 2C), 127.68, 127.10, 122.31, 118.22, 116.19, 115.98, 111.87, 41.86 ppm. HRMS (ESI) m/z calcd for $[M-H]^-$ 324.08, found 324.0660.

(E)-2-(5-(4-chlorostyryl)-2,3-dioxoindolin-1-yl)acetic acid (9c) The reaction was carried out according to the general procedure F using compound **8c** (192.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **9c** as red powder (111 mg, 65%). M.p. 274–276 °C; purity: 96.86%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.23 (s, 1H), 7.90 (d, $J = 9.4$ Hz, 2H), 7.61 (d, $J = 8.5$ Hz, 2H), 7.44 (d, $J = 8.5$ Hz, 2H), 7.31 (s, 2H), 7.24 (d, $J = 8.1$ Hz, 1H), 4.53 (s, 2H).^{13}C NMR (101 MHz, DMSO-d_6) δ 183.31, 169.28, 158.79, 150.18, 136.84, 136.37, 133.22, 132.44, 129.19 (2C), 128.56 (2C), 128.08, 127.51, 122.47, 118.24, 111.89, 41.83 ppm. HRMS (ESI) m/z calcd for $[M + H]^+$ 342.05, found 342.0524.

(E)-2-(2,3-dioxo-5-(4-(trifluoromethyl)styryl)indolin-1-yl) acetic acid (9d) The reaction was carried out according to the general procedure F using compound **8d** (209.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **9d** as red powder (120 mg, 65%). M.p. 270–272 °C; purity: 96.90%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.30 (s, 1H), 8.10 (dd, $J = 8.3$, 1.9 Hz, 1H), 8.07–7.89 (m, 4H), 7.80 (dd, $J = 17.6$, 9.9 Hz, 3H), 7.36 (d, $J = 8.3$ Hz, 1H), 4.58 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.27, 169.28, 158.80, 150.50,141.56, 137.17, 132.88, 130.15, 128.15, 127.84, 127.41 (2C), 127.23, 126.07 (d, $J = 3.7$ Hz, 2C), 122.76, 118.27, 111.93, 41.86 ppm. HRMS (ESI) m/z calcd for $[M + H]^+$ 376.07, found 376.0795.

(E)-2-(5-(4-methylstyryl)-2,3-dioxoindolin-1-yl)acetic acid (9e) The reaction was carried out according to the general procedure F using compound **8e** (183.5 mg, 0.5 mmol), concentrated HCl

(2 mL) and THF (8 mL) to give **9e** as red powder (101 mg, 68%). M.p. 266–268 °C; purity: 99.95%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.26 (s, 1H), 7.91–7.85 (m, 2H), 7.48 (d, J = 7.4 Hz, 2H), 7.26 (s, 1H), 7.23–7.18 (m, 4H), 4.53 (s, 2H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.39, 169.29, 158.79, 149.90, 137.61, 136.54, 134.59, 133.65, 129.80, 128.81, 126.89, 126.17, 122.27, 118.21, 111.85, 41.81, 21.33 ppm. HRMS (ESI) m/z calcd for $[M + H]^+$ 322.10, found 322.1063.

(E)-2-(5-(4-(tert-butyl)styryl)-2,3-dioxoindolin-1-yl)acetic acid (9f) The reaction was carried out according to the general procedure F using compound **8f** (203.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **9f** as red powder (98 mg, 65%). M.p. 252–254 °C; purity: 96.54%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.25 (s, 1H), 7.90 (d, J = 8.3 Hz, 1H), 7.87 (s, 1H), 7.52 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 8.3 Hz, 2H), 7.29 (d, J = 16.5 Hz, 1H), 7.24–7.21 (m, 2H), 4.53 (s, 2H), 1.29 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.38, 169.29, 158.79, 150.81, 149.92, 136.56, 134.60, 133.65, 128.70, 126.71 (2C), 126.36, 125.95 (2C), 122.32, 118.21, 111.86, 41.81, 34.83, 31.53 (3C) ppm. HRMS (ESI) m/z calcd for $[M + H]^+$ 364.15, found 364.1531.

(E)-2-(5-(4-methoxystyryl)-2,3-dioxoindolin-1-yl)acetic acid (9g) The reaction was carried out according to the general procedure F using compound **8g** (190.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **9g** as red powder (109 mg, 65%). M.p. 250–252 °C; purity: 99.23%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.26 (s, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.83 (s, 1H), 7.53 (d, J = 8.6 Hz, 2H), 7.23–7.20 (m, 2H), 7.11 (d, J = 16.5 Hz, 1H), 6.96 (d, J = 8.6 Hz, 2H), 4.53 (s, 2H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.43, 169.29, 159.52, 158.78, 149.69, 136.34, 133.90, 130.00, 128.57, 128.27 (2C), 124.86, 122.06, 118.20, 114.67 (2C), 111.83, 55.63, 41.80 ppm. HRMS (ESI) m/z calcd for $[M\text{-}H]^-$ 336.10, found 336.0878.

(E)-2-(5-(4-hydroxy-3-methoxystyryl)-2,3-dioxoindolin-1-yl) acetic acid (9h) The reaction was carried out according to the general procedure F using compound **8h** (198.5 mg, 0.5 mmol), concentrated HCl (2 mL) and THF (8 mL) to give **9h** as red powder (114 mg, 65%). M.p. 220–222 °C; purity: 99.94%, ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.14 (s, 1H), 9.17 (s, 1H), 7.84 (d, J = 8.3 Hz, 1H), 7.81 (s, 1H), 7.21–7.18 (m, 4H), 7.11 (s, 1H), 6.79–6.77 (m, 1H), 4.51 (s, 2H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 183.52, 169.38, 158.77, 149.58, 148.29, 147.26, 136.16, 134.06, 129.26, 128.96, 124.07, 121.85, 120.71, 118.21, 116.05, 111.86, 110.32, 56.05, 41.87 ppm. HRMS (ESI) m/z calcd for $[M + H]^+$ 354.09, found 354.0959.

Enzyme assays

Wistar rats (200–250 g) used in the experiment were supplied by SPF (Beijing) Biotechnology Co., Ltd. (certificate: 110229013482187, Beijing, China). According to the eported methods of Kinoshita J [33] and La Motta C [7], ALR1 and ALR2 were extracted from rat kidney and lens, respectively.

The ALR2 inhibition activity was tested in a sample solution containing sodium phosphate buffer (0.25 mL, 0.1 M, pH 6.2), enzyme extract (0.1 mL), deionized water (0.15 mL) and NADPH (0.25 mL, 0.10 mM). Appropriate blanks were used for corrections, which contains sodium phosphate buffer (0.50 mL, 0.1 M, pH 6.2), enzyme extract (0.1 mL), deionized water (0.15 mL), NADPH (0.25 mL, 0.10 mM) and D, L-glycerol Aldehydes (0.25 mL, 10 mM) and 0.5% dimethyl sulfoxide as solvent. The sample solution was kept at 30 °C for 10 min and the substrate (D,L-glyceraldehyde 0.25 mL, 10 mM) was then added to start the reaction. The absorption reduction rate of NADPH at λ 340 nm was monitored for 5 min.

The ALR1 inhibition activity was tested in a sample solution containing sodium phosphate buffer (0.25 mL, 0.1 M, pH 7.2), enzyme extract (0.1 mL), NADPH (0.25 mL, 0.12 mM), deionized water (0.15 mL). Appropriate blanks were used for corrections, which contains sodium phosphate buffer (0.25 mL, 0.1 M, pH 7.2), enzyme extract (0.1 mL), NADPH (0.25 mL, 0.12 mM), deionized water (0.15 mL), sodium D-glucuronate (0.25 mL, 20 mM) and 0.5% dimethyl sulfoxide as solvent. The sample solution was kept at 36 °C for 10 min and the substrate (sodium D-glucuronate 0.25 ml, 20 mM) was then added to start the reaction. The absorption reduction rate of NADPH at λ 340 nm was monitored for 5 min.

Compound solutions were prepared by dissolving the compounds in dimethyl sulfoxide (DMSO) and diluting to different concentrations. 5 μL of the compound solution was added to the above reaction mixture. To correct for the non-enzymatic oxidation of NADPH, the oxidation rate of NADPH in the presence of all reaction mixture components except the substrate was subtracted from each experimental rate. Firstly, the inhibitory effect of synthetic compounds was determined at a concentration of 100 μM (concentration in the final reaction system). Secondly, the inhibitory effect at other concentrations (between 100 μM and 10 nM) was determined. 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. Lastly, IC_{50} values were calculated by linear regression analysis and regression equation ($r^2 > 0.95$).

DPPH assays

A method based on the DPPH scavenging of stable free radicals was used to investigate the antiradical activity of the tested compounds in a homogeneous system [34]. 100 µL of methanol solutions of various compounds (or Trolox) with different concentrations were added to the tube containing 1 mL of DPPH methanol solution (0.025 mg/mL) and 1.9 mL of methanol solution, so that the final concentrations of the tested compounds were 100, 10 and 5 µM, respectively. The composition of the control group was DPPH methanol solution (1 mL, 0.025 mg/mL) and methanol (2 mL). The methanol solution (0.1 mL) of the testing compound and methanol (2.9 mL) constituted a blank group. The above reaction system were vortexed thoroughly and placed at room temperature for 120 min. The absorbance was measured at λ 517 nm by the Shimadzu UV-1800 spectrophotometer. The percentage of DPPH radical scavenging was calculated by the following equation. Experiments were performed in triplicate.

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

Lipid peroxidation inhibition

The brain homogenate was prepared by crushing freshly isolated rat brains with ice-cold normal saline. Then the homogenate was centrifuged at a speed of 3000 rpm for 10 min to obtain the supernatant for biochemical analysis. The protein concentration in the supernatant was determined by a total protein quantification kit. 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. After that, TBA was mixed with the reaction mixture and then centrifuged to obtain supernatant. The concentration of MDA in the supernatant was determined by a commercially available TBA-based kit. All the subsequent steps were followed according to the instruction of manufacturer [35, 36]. The supernatant and solution in the MDA kit were mixed in a centrifuge tube and boiled at 95 °C for 40 min. Then, the centrifuge tubes were cooled and centrifuged (4000 rpm, 10 min). The red product produced by the reaction was measured with a spectrophotometer at λ 532 nm. Experiments were performed in triplicate.

Docking studies

Docking was performed by Molegro Virtual Docker (version 5.0) with the same protocol as described before [23].

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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References

1. Hao X, Han Z, Li Y, Li C, Wang X, Zhang X, et al. Synthesis and structure–activity relationship studies of phenolic hydroxyl derivatives based on quinoxalinone as aldose reductase inhibitors with antioxidant activity. Bioorg Med Chem Lett. 2017;27:887–92
2. Maccari R, Ottanà R. Targeting aldose reductase for the treatment of diabetes complications and inflammatory diseases: new insights and future directions. J Med Chem. 2015;58:2047–67
3. Alexiou P, Pegklidou K, Chatzopoulou M, Nicolaou I, Demopoulos VJ. Aldose reductase enzyme and its implication to major health problems of the 21st century. Curr Med Chem. 2009;16:734–52
4. Chen H, Zhang X, Zhang X, Fan Z, Liu W, Lei Y, et al. Dihydrobenzoxazinone derivatives as aldose reductase inhibitors with antioxidant activity. Bioorg Med Chem. 2020;28:115699
5. Quattrini L, La Motta C. Aldose reductase inhibitors: 2013-present. Expert Opin Ther Pat. 2019;29:199–213
6. Kador PF. The role of aldose reductase in the development of diabetic complications. Med Res Rev. 1988;8:325–52
7. La Motta C, Sartini S, Mugnaini L, Simorini F, Taliani S, Salerno S, et al. Pyrido[1,2-a]pyrimidin-4-one derivatives as a novel class of selective aldose reductase inhibitors exhibiting antioxidant activity. J Med Chem. 2007;50:4917–27
8. Han Z, Hao X, Ma B, Zhu C. A series of pyrido[2,3-b]pyrazin-3(4H)-one derivatives as aldose reductase inhibitors with antioxidant activity. Eur J Med Chem. 2016;121:308–17
9. Da Settimo F, Primofiore G, La Motta C, Sartini S, Taliani S, Simorini F, et al. Naphtho [1, 2-d] isothiazole acetic acid derivatives as a novel class of selective aldose reductase inhibitors. J Med Chem. 2005;48:6897–907
10. Maccari R, Vitale RM, Ottanà R, Rocchicciol M, Marrazzo A, Cardile V, et al. Structure-activity relationships and molecular modelling of new 5-arylidene-4-thiazolidinone derivatives as aldose reductase inhibitors and potential anti-inflammatory agents. Eur J Med Chem. 2014;81:1–14
11. Wiernsperger NF. Oxidative stress as a therapeutic target in diabetes: revisiting the controversy. Diabetes Metab. 2003;29:579–85
12. Farooqui AA. Inflammation and oxidative stress in neurological disorders. Springer International Publishing, 2014
13. Tang WH, Martin KA, Hwa J. Aldose reductase, oxidative stress, and diabetic mellitus. Front Pharm. 2012;3:87
14. Drel VR, Pacher P, Ali TK, Shin J, Julius U, El-Remessy AB, et al. Aldose reductase inhibitor fidarestat counteracts diabetes-associated cataract formation, retinal oxidative-nitrosative stress, glial activation, and apoptosis. Int J Mol Med. 2008;21:667–76
15. Drel VR, Pacher P, Stevens MJ, Obrosova IG. Aldose reductase inhibition counteracts nitrosative stress and poly (ADP-ribose) polymerase activation in diabetic rat kidney and high-glucose-exposed human mesangial cells. Free Radic Biol Med. 2006;40:1454–65
16. Ji Y, Chen X, Chen H, Zhang X, Fan Z, Xie L, et al. Designing of acyl sulphonamide based quinoxalinones as multifunctional aldose reductase inhibitors. Bioorg Med Chem. 2019;27:1658–69

17. Hamada Y, Araki N, Horiuchi S, Hotta N. Role of polyol pathway in nonenzymatic glycation. Nephrol, Dial, Transpl. 1996;11:95–98
18. Chung SSM, Chung SK. Genetic analysis of aldose reductase in diabetic complications. Curr Med Chem. 2003;10:1375–87
19. Van Zandt MC, Jones ML, Gunn DE, Geraci LS, Jones JH, Sawicki DR, et al. Discovery of 3-[(4, 5, 7-trifluorobenzothiazol-2-yl) methyl] indole-N-acetic acid (lidorestat) and congeners as highly potent and selective inhibitors of aldose reductase for treatment of chronic diabetic complications. J Med Chem. 2005;48:3141–52
20. Mylari BL, Armento SJ, Beebe DA, Conn EL, Coutcher JB, Dina MS, et al. A highly selective, non-hydantoin, non-carboxylic acid inhibitor of aldose reductase with potent oral activity in diabetic rat models: 6-(5-chloro-3-methylbenzofuran-2-sulfonyl)-2-H-pyridazin-3-one. J Med Chem. 2003;46:2283–6
21. Steele JW, Faulds D, Goa KL. Epalrestat. Drugs Aging. 1993;3:532–55
22. Maccari R, Ottanà R. 2, 4-Thiazolidinedione and 2-thioxo-4-thiazolidinone derivatives as aldose reductase inhibitors in the search for drugs to prevent long-term diabetes complications. Adv Mol Mech Pharmacol Diabetic Complications. 2010;219–45
23. Qin X, Hao X, Han H, Zhu S, Yang Y, Wu B, et al. Design and synthesis of potent and multifunctional aldose reductase inhibitors based on quinoxalinones. J Med Chem. 2015;58:1254–67
24. Barski OA, Gabbay KH, Grimshaw CE, Bohren KM. Mechanism of human aldehyde reductase: characterization of the active site pocket. Biochemistry. 1995;34:11264–75
25. Wang J, Yun D, Yao J, Fu W, Huang F, Chen L, et al. Design, synthesis and QSAR study of novel isatin analogues inspired michael acceptor as potential anticancer compounds. Eur J Med Chem. 2018;144:493–503
26. Zhang Q, Teng Y, Yuan Y, Ruan T, Wang Q, Gao X, et al. Synthesis and cytotoxic studies of novel 5-phenylisatin derivatives and their anti-migration and anti-angiogenic evaluation. Eur J Med Chem. 2018;156:800–14
27. Andreani A, Burnelli S, Granaiola M, Leoni A, Locatelli A, Morigi R, et al. New isatin derivatives with antioxidant activity. Eur J Med Chem. 2010;45:1374–8
28. Zuo A-R, Dong H-H, Yu Y-Y, Shu Q-L, Zheng L-X, Yu X-Y, et al. The antityrosinase and antioxidant activities of flavonoids dominated by the number and location of phenolic hydroxyl groups. Chin Med. 2018;13:1–12
29. Wang C, White A, Schwartz S, Alluri S, Cattabiani T, Zhang L, et al. Novel synthesis and functionalization of ortho–ortho disubstituted biphenyls and a highly condensed novel heterocycle using radical cyclization reaction. Tetrahedron. 2012;68:9750–62
30. Joule JA. Thiophenes from viktor meyer to poly (thiophene) some reactions and synthesis. Phosphorus Sulfur Silicon Relat Elem. 2013;188:287–316
31. Zhou N, Polozov AM, O'Connell M, Burgeson J, Yu P, Zeller W, et al. 1,7-Disubstituted oxyindoles are potent and selective EP 3 receptor antagonists. Bioorg Med Chem Lett. 2010;20:2658–64
32. El-Kabbani O, Ramsland P, Darmanin C, Chung RPT, Podjarny A. Structure of human aldose reductase holoenzyme in complex with Statil: An approach to structure-based inhibitor design of the enzyme. Proteins: Struct, Funct, Bioinf. 2003;50:230–8
33. Hayman S, Kinoshita JH. Isolation and properties of lens aldose reductase. J Biol Chem. 1965;240:877–82
34. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1199–200
35. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351–8
36. Liu L, Liu Y, Cui J, Liu H, Liu Y-B, Qiao W-L, et al. Oxidative stress induces gastric submucosal arteriolar dysfunction in the elderly. World J Gastroenterol. 2013;19:9439–46