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Short communication

Novel 3,4-dihydroquinolin-2(1*H*)-one derivatives as dual inhibitor targeting AKR1B1/ROS for treatment of diabetic complications: Design, synthesis and biological evaluation

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ABSTRACT

AKR1B1 (Aldose reductase) has been used as therapeutic intervention target for treatment of diabetic complications over 50 years, and more recently for inflammation and cancer. However, most developed small molecule inhibitors have the defect of low bioactivity. To address this limitation, novel series of 3,4-dihydroquinolin-2(1*H*)-one derivatives as dual inhibitor targeting AKR1B1/ROS (Reactive Oxygen Species) were designed and synthesized. Most of these derivatives were found to be potent and selective against AKR1B1, and compound **8a** was the most active with an IC_{50} value of 0.035 μM. Moreover, some prepared derivatives showed strong anti-ROS activity, and among them the phenolic 3,5-dihydroxyl compound **8b** was proved to be the most potent, even comparable to that of the well-known antioxidant Trolox at a concentration of 100 μM. Thus the results suggested a success in the construction of potent dual inhibitor for the therapeutic intervention target of AKR1B1/ROS.

1. Introduction

Diabetes Mellitus (DM), companied with cardiovascular and cancer, are recognized as the three most mainly reasons lead to human death. Based on latest statistical data from American Diabetes Association (ADA), over 256 million people worldwide were suffering diabetes mellitus and this figure would increase steadily to 366 million by 2030 [1]. All form of DM, including type I and type II, are characterized by hyperglycaemia and vulnerable to chronic diabetic complications, such as nephropathy, cataracts and retinopathy. These diabetic complications are the main threaten and primary reason of death to diabetic patients [2,3]. Numerous evidences have demonstrated that aldose reductase (AKR1B1, EC1.1.1.21) served as a pivotal factor for the onset and progression of chronic diabetic complications. AKR1B1 catalyzes the reduction of glucose to sorbitol in the rate-limiting step of the polyol pathway (Fig. 1), following with sorbitol degydrogenase (SDH) oxidizes sorbitol to fructose [4]. This glucose metabolism pathway is considered as the leading pathogenesis of the diabetic complications. Normally, the AKR1B1 has poor activity and only 3% of glucose metabolize through this pathway. However, under hyperglycemia, the activity of AKR1B1 is stimulated and this figure could rise up to about 33% in tissues demonstrating insulin-independent uptake of glucose, such as lens, kidney, retina, and peripheral nerves [5–7]. The increased glucose metabolism through polyol pathway directly results in various cellular stress conditions, which provide the underlying mechanism of chronic diabetic complications [8,9]. Furthermore, AKR1B1 was identified as the major mediator in the pathologies of inflammation, which are related to various types of cancers (colon, breast, lung e.g.) [10]. As showed in Fig. 1, the physiological role of AKR1B1 is the key step in the manifestation and propagation of Reactive Oxygen Species (ROS) induced inflammation. 4-hydroxy-2-nonenal (HNE), a product of lipid peroxidation, and its glutathione adduct (GS-HNE) are converted by AKR1B1 to 1,4-dihydroxynonene (DHN) and GS-DHN respectively. A cascade of kinases (PLC, PKC, MAPK) and transcription factors (NF-κB, AP-1) are in turn active, leading to production of pro-inflammatory signals. Therefore, AKR1B1 and ROS are considered to be the two most important intervention targets for diabetic complications [11].

A number of various AKR1B1 inhibitors (Fig. 2) have been developed

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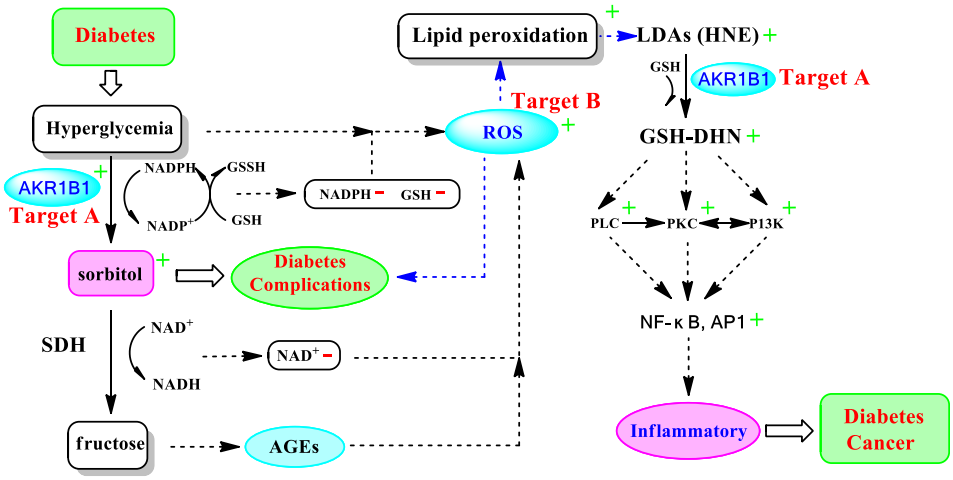


Fig. 1. Polyol pathway of glucose metabolism.

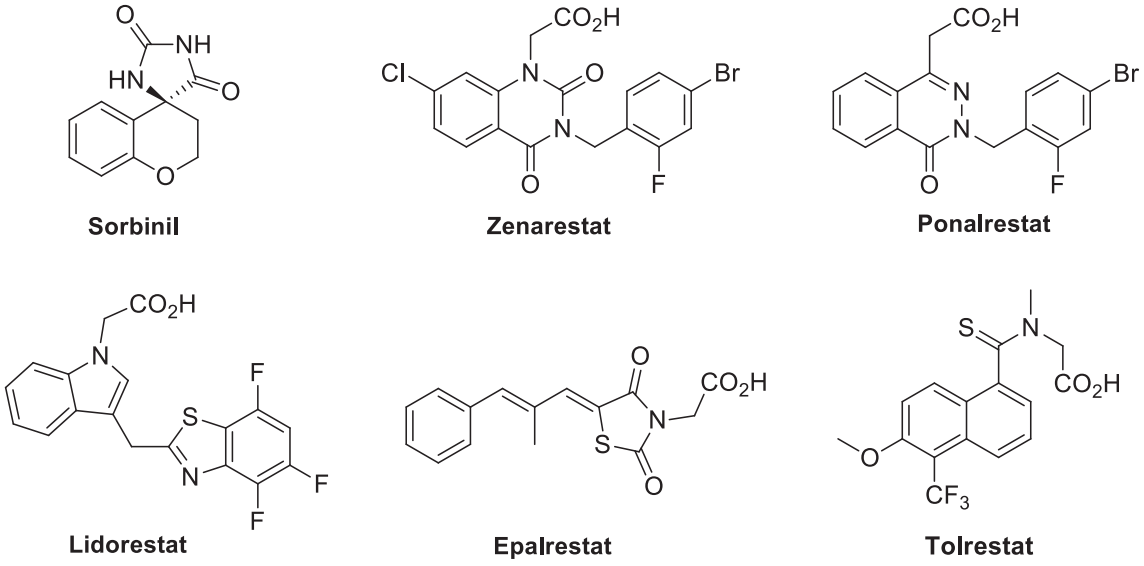


Fig. 2. Structures of ARIs.

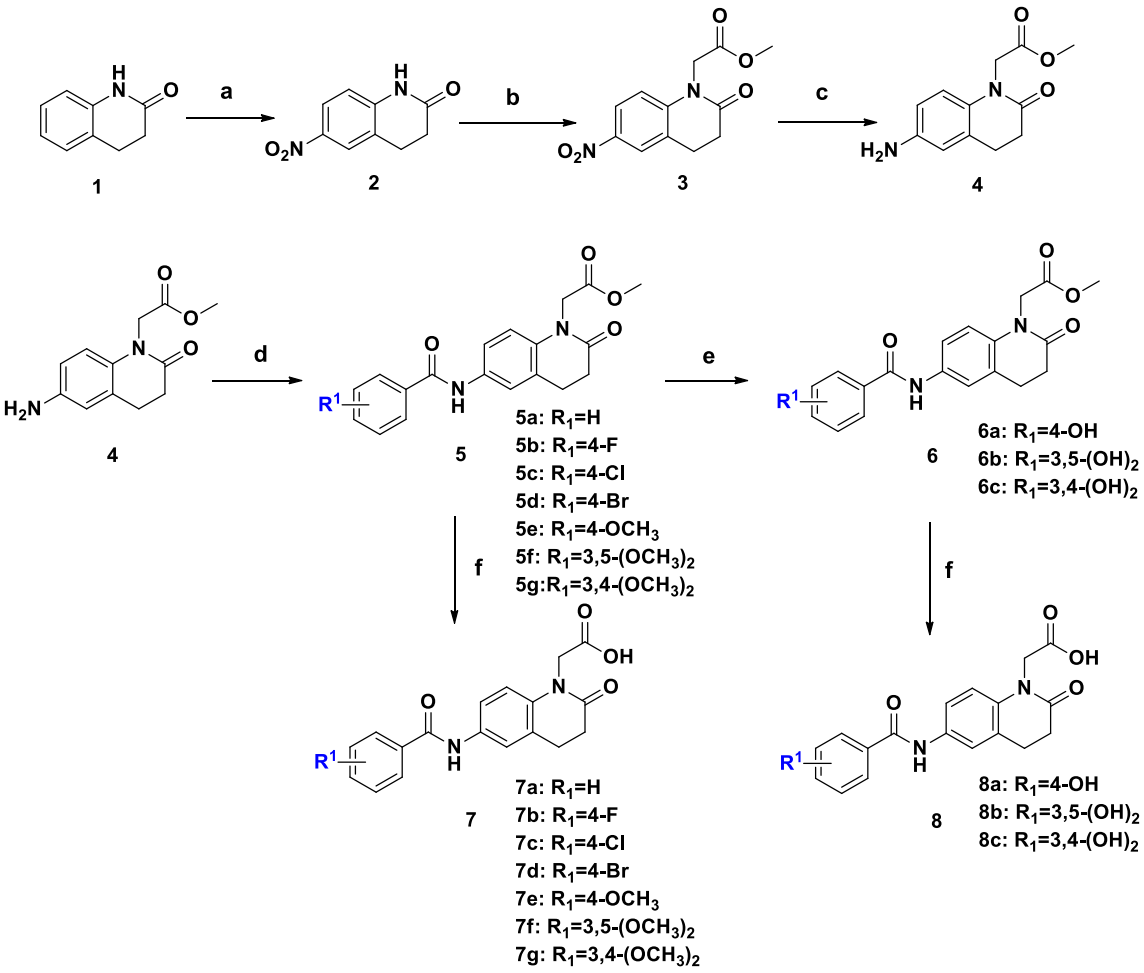
and some of them are endowed with excellent inhibitory activity [12,13]. However, most of the AKR1B1 inhibitors (ARIs) were failed clinically due to inadequate efficacies or pharmacokinetic drawbacks. Although the underlying mechanism of the low efficacy is unclear at present, it is speculated that only inhibiting one target AKR1B1 is not enough to prevent and treat pathological changes in all the tissues of diabetic patients. Based on the pathogenesis of diabetic complications, developing dual inhibitor targeting AKR1B1 and ROS which keeps the AKR1B1 in its reduced form and concurrently decreases the tissue damage due to ROS would be potential therapeutic strategies for treatment of diabetic complications.

We have prepared several groups of AKR1B1 inhibitors with potential inhibitory activity previously, but most of them were not endowed with the anti-ROS activity [14–19]. In this study, the anti-ROS activity was introduced to a new core structure of 3,4-dihydroquinolin-2(1*H*)-one, which was an excellent core structure applied in many drug molecules design, such as protein 1 kinases inhibitors, renin inhibitors, modulating peroxisome proliferator activated receptor (PPAR) activity, serine protease inhibitor and recently antioxidant [20]. Thus a series dual inhibitors based on 3,4-dihydroquinolin-2(1*H*)-one were developed targeting AKR1B1 and ROS.

2. Results and discussion

2.1. Synthesis

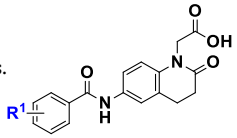
The commercially available 3,4-dihydroquinolin-2(1*H*)-one was used as the starting material, which was treated with a mixture of concentrated sulfuric acid and nitric acid (4:1) under ice cooling to give compound **2**. Compound **2** was alkylated at the N1 position with methyl bromoacetate to form methyl ester **3**, and then the nitro group at the 6-position of compound **3** was reduced with palladium on activated carbon under hydrogen atmosphere to afford the corresponding amino compound **4** with a high yield. The 6-position of compound **4** was acylated with different benzoyl chloride to form compound **5** as key intermediate for obtaining various target compounds (**7** and **8**). Hydrolysis of substituted compounds **5a-g** with lithium hydroxide obtained the target carboxylic acid compounds **7a-g**, and demethylation of the methoxyl with $AlCl_3$ followed by hydrolysis of **5e**, **5f** and **5g** gave the target compounds **8a-c** (see Scheme 1). The detailed experimental data was depicted in the supplemental material.



Scheme 1. **a**: HNO_3, H_2SO_4, −10 °C; **b**: $BrCH_2COOCH_3$, NaH, THF, r.t.; **c**: H_2, Pd/C, r.t.; **d**: Benzoyl chloride derivative, DMF, Et_3N; **e**: $AlCl_3$, CH_2Cl_2, 45 °C; **f**: 1) LiOH, THF, H_2O, r.t., 2) HCl.

Table 1

Biological activity of 3,4-dihydroquinolin-2(1*H*)-one derivatives.



No.	Substituent	IC_{50}(μM)[a]	IC_{50}(μM)[a]	Ratio	DPPH sca.%		
	R^1	AKR1B1	AKR1A1	AKR1A1/AKR1B1	100 μM	50 μM	10 μM
7a	H	12.187 ± 1.114	148.277 ± 1.354	12.2	20.3 ± 0.7	*	*
7b	4-F	0.107 ± 0.009	19.187 ± 1021	179.3	19.7 ± 0.5	*	*
7c	4-Cl	0.231 ± 0.013	20.147 ± 1.311	87.2	21.7 ± 0.6	*	*
7d	4-Br	0.358 ± 0.074	24.871 ± 1.647	69.5	19.8 ± 0.9	*	*
7e	4-OCH_3	7.047 ± 0.574	80.188 ± 1.508	11.4	39.3 ± 0.7	*	*
7f	3,5-$(OCH_3)_2$	9.217 ± 0.824	121.617 ± 2.874	13.2	45.1 ± 0.8	*	*
7g	3,4-$(OCH_3)_2$	10.421 ± 0.918	107.872 ± 0.879	10.4	50.8 ± 1.3	*	*
8a	4-OH	0.035 ± 0.006	15.214 ± 0.911	434.6	86.9 ± 0.9	75.8 ± 0.6	45.2 ± 0.5
8b	3,5-$(OH)_2$	0.042 ± 0.002	18.103 ± 1.014	431.0	94.1 ± 1.1	81.4 ± 0.9	69.6 ± 0.8
8c	3,4-$(OH)_2$	0.097 ± 0.004	19.547 ± 0.987	201.5	90.5 ± 0.7	77.5 ± 0.9	58.1 ± 0.7
Epalrestat	–	0.074 ± 0.008	48.217 ± 2.579	651.6	*	*	*
Trolox	–	*	*	*	96.2 ± 1.3	85.3 ± 1.1	78.5 ± 0.9

[a] IC_{50} (95% CL) values represent the concentration of the tested compounds required to decrease enzymatic activity by 50%.
* Not test.

2.2. Inhibition of enzymes

All synthetic target compounds **7a-g** and **8a-c** were tested for their potential inhibitory activity of AKR1B1 isolated from rat lenses, and their selectivity for the AKR1B1 inhibition were tested by the identification of inhibitory activity against the enzyme of aldehyde reductase (AKR1A1, EC1.1.1.2) extracted from rat kidneys. AKR1A1 is closely related to AKR1B1, and plays an important role of physiological

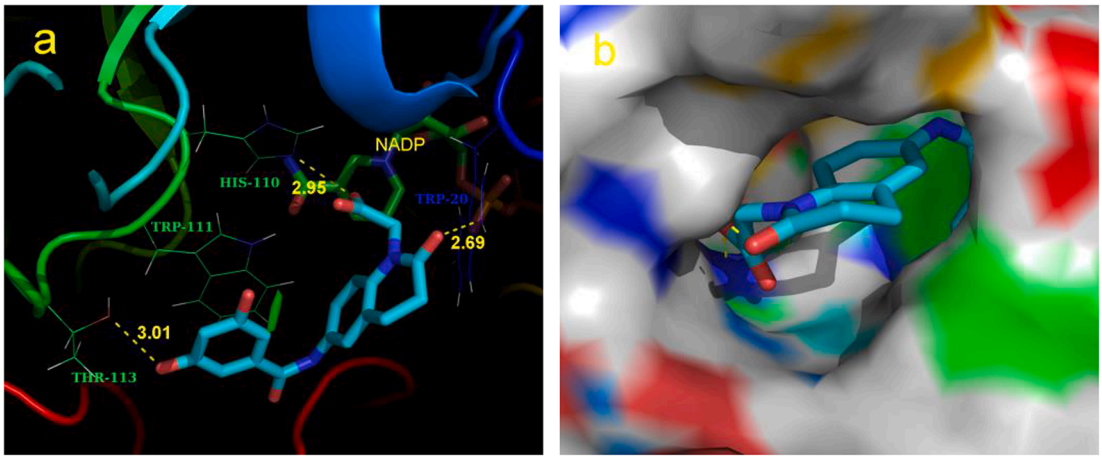


Fig. 3. Docking of the inhibitor **8b** into the active site of AKR1B1. **(a)** The protein structure is shown as a cartoon diagram with selected residues labeled and shown in line representation, ligand and NADP are shown as stick models. The docked pose of **8b** is shown in cyan (C), red (O) and blue (N). Hydrogen bonds are shown as yellow dashed lines. **(b)** Protein residues are in surface representation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

detoxification. The results are expressed as IC_{50} (μM) summarized in Table 1.

As shown in Table 1, most of the 3,4-dihydroquinolin-2(1*H*)-one derivatives showed significant AKR1B1 inhibition and selectivity. Of all the compounds, 2-(6-(4-hydroxybenzamido)-2-oxo-3,4-dihydroquinolin-1(2*H*)-yl)acetic acid **8a** was the most active with an IC_{50} value of 0.035 μM, and it was more potent than the positive control epalrestat. In compounds **7a-g**, it was found that the introduction of halogen atom or methoxyl to the C6-aryl side chain of **7a** could enhance the inhibitory activity, and halogen atom showed better enhancement. Compounds **7e**, **7f** and **7 g** having one or two methoxyl on the C6-aryl side chain displayed relatively low AKR1B1 inhibition with IC_{50} values ranging from 7.047 to 10.421 μM, compared with compounds **7b**, **7c** and **7d** having halogen atom on the corresponding position of aryl side chain. Moreover, the effect of halogen substituent on AKR1B1inhibition was in the rank order of 4-F > 4-Cl > 4-Br and the corresponding IC_{50} values were 0.107 μM, 0.231 μM and 0.358 μM, respectively. Besides, it was encouraging to find that the inhibitory activity of AKR1B1 was enhanced when the methoxy was replaced to hydroxyl group, which was concluded by comparing the inhibitory activity of compounds **7e-g** and **8a-c**. Particularly, compound **8b** having two phenolic hydroxyl groups on C6-aryl side chain were endowed with significant AKR1B1 inhibitory activity with an IC_{50} value of 0.042 μM. Meanwhile, all target compounds were also evaluated for their inhibition ability against AKR1A1, and showed low activity with IC_{50} values more than 15.214 μM, demonstrating good selectivity for the AKR1B1 inhibition.

2.3. The antioxidant activity

The anti-ROS properties of the synthesized compounds were also investigated in the present work by using the model reaction with the stable free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the modified method [21], and 6-hydroxy-2,5,7,8-chroman-2-carboxylic acid (Trolox) was employed as a positive control. As shown in Table 1, all derivatives showed DPPH radical scavenging activity ranging from 19.7 to 94.1% at the concentration of 100 μM. Of all tested compounds, **8b** with phenolic 3,5-dihydroxyl on the C6-aryl ring showed the best scavenging activity, that is, 94.1%, 81.4%, and 69.6% at concentrations of 100 μM, 50 μM and 10 μM respectively, which had an commensurate activity compared with Trolox at high concentrations. Structure-activity relationship (SAR) study of compounds (**7e** vs **8a**, **7f** vs **8b** and **7 g** vs **8c**) indicated that demethylation of the methoxyl on C6-aryl side chain could improve the radical scavenging activity significantly. The number and position of phenolic hydroxyl also had effect on activity, and the phenolic 3,5-dihydroxyl substituent was the most effective on activity enhancement when comparing all the phenolic hydroxyl derivatives. Moreover, comparison of compounds **7b-d** with **7a** revealed that the halogen substituent had little impact on the radical scavenging activity.

2.4. Molecular docking

To better understand the mechanistic details and the above-described SARs, compound **8b** with excellent activities both in the AKR1B1 inhibition and anti-ROS activity was docked with the conformation of the human AKR1B1/$NADP^+$/lidorestat complex (PDB code: 1Z3N). As shown in Fig. 3, compound **8b** was tightly bound into the active site of AKR1B1. The carboxylate group was inserted deeply in the anion binding site by forming tight hydrogen-bonding interaction with the side chain of His110 (2.95 Å). Besides, the 3-hydroxyl oxygen atom of the phenolic hydroxyl on C6-aryl side chain formed an additional hydrogen bond with the side chain of Thr113 (3.01 Å), confirming the importance of phenolic hydroxyl on the activity enhancement of AKR1B1 inhibition. Moreover, the 3,5-dihydroxyphenyl ring of the C6 side chain was well placed into the specificity pocket and paralleled to the indole ring of Trp111 forming a stable stacking interaction. Meanwhile, the 3,4-dihydroquinolin-2(1*H*)-one core structure matched very well the hydrophobic pocket and a tight hydrogen-bonding interaction with Trp20 (2.69 Å) was formed, which indicates that the 3,4-dihydroquinolin-2(1*H*)-one is a potent core structure for developing AKR1B1 inhibitor. All these interactions anchor the inhibitor **8b** tightly within the active site of AKR1B1.

3. Conclusion

In conclusion, a series of inhibitor candidates based on a novel core structure 3,4-dihydroquinolin-2(1*H*)-one were synthesized and biologically evaluated for AKR1B1 inhibition and anti-ROS properties. The biological results showed that all compounds exhibited excellent AKR1B1 inhibitory activity with IC_{50} values ranging from 0.035 to 12.187 μM, and 2-(6-(4-hydroxybenzamido)-2-oxo-3,4-dihydroquinolin-1(2*H*)-yl)acetic acid **8a** was the most active. Compounds **8a**, **8b** and **8c** containing phenolic hydroxyl on the C6-aryl side chain were not only sufficient to inhibit AKR1B1 but also effective for DPPH radical scavenging, which indicated success in the development of dual inhibitor targeting for AKR1B1 and ROS. Therein, compound **8b** with phenolic 3,5-dihydroxyl on the C6-aryl ring showed the best DPPH

scavenging activity even comparable with Trolox at high concentrations. Further SAR and molecular docking studies highlighted the importance of N1-acetic acid head group along with phenolic hydroxyl substituent in C6-aryl side chain of the scaffold for construction of potent and multi-effective dual inhibitor.

Declaration of Competing Interest

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.104428.

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