Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: http://www.tandfonline.com/loi/ienz20

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To cite this article: Shaojuan Zhu, Shuzhen Zhang, Xin Hao, Xiangyu Qin, Shagufta Parveen, Shaoqi Yang, Bing Ma & Changjin Zhu (2016): Pyridothiadiazine derivatives as aldose reductase inhibitors having antioxidant activity, Journal of Enzyme Inhibition and Medicinal Chemistry, DOI: 10.1080/14756366.2016.1178638

To link to this article: http://dx.doi.org/10.1080/14756366.2016.1178638

Journal of Enzyme Inhibition and Medicinal Chemistry

www.tandfonline.com/ienz
ISSN: 1475-6366 (print), 1475-6374 (electronic)

J Enzyme Inhib Med Chem, Early Online: 1–5
 DOI: 10.1080/14756366.2016.1178638

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ORIGINAL ARTICLE

Pyridothiadiazine derivatives as aldose reductase inhibitors having antioxidant activity

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Abstract

A series of aldose reductase (ALR2) inhibitors based on pyridothiadiazine were prepared and evaluated for their activities in ALR2 inhibition, DPPH scavenging, and MDA inhibition. Comparison studies were carried out between analogs having either hydroxyl or methoxy groups substituted on the N2-benzyl side chains of the compounds. Most of the hydroxy-substituted compounds were found to be more potent compared to their methoxy-substituted analogs with respect to DPPH inhibition (>93%) and MDA inhibition (>73%). However, ALR2 inhibitory activity was found to be affected by the electron-withdrawing substituent at the C7 position in addition to the effect of the N2-substituted benzyl group. These results provide an array of multifunctional ALR2 inhibitors possessing capacities both for ALR2 inhibition and as antioxidants.

Keywords

Aldose reductase inhibitors, antioxidant activity, pyridothiadiazine derivatives, structure-activity relationships

History

Received 30 January 2016
Revised 12 March 2016
Accepted 14 March 2016
Published online 3 May 2016

Introduction

Diabetes mellitus (DM), a life-threatening disease distributed worldwide, is associated with various complications such as retinopathy, nephropathy, cataract, neuropathy and stroke[1,2]. Diabetic complications triggered by hyperglycemia have been proposed to involve three major pathways: protein kinase C (PKC) isoform activation, increased glucose flux via the aldose reductase pathway and higher production of glucose-derived advanced glycation end products (AGE). As the starting event of DM development is hyperglycemia, mechanisms such as glucose-stimulated free radical production and hyperglycemia-induced overproduction of mitochondrial superoxide have also been studied[3–5]. Under hyperglycemic conditions, the polyol metabolic pathway is activated (Figure 1) and leads to the accumulation of sorbitol. Aldose reductase (E.C.1.1.1.21, ALR2), as the first and rate-determining enzyme, catalyzes the reduction of excess glucose to sorbitol in the presence of co-factor NADPH. In turn, sorbitol dehydrogenase converts sorbitol to fructose through an NAD^+-dependent reaction. Sorbitol accumulation causes osmotic stress, which results in tissue damage. Furthermore, the increase in the NADH/NAD^+ ratio promotes the activation of PKC isoform that are believed to act as mediators for biochemical and functional alterations induced by hyperglycemia. Moreover, the activity of the NADPH-requiring enzyme glutathione (GSH) reductase decreases with the depletion of NADPH cofactor. The decrease in GSH level is associated with a concomitant rise in toxic lipid peroxidation products like malondialdehyde (MDA) and trans-4-hydroxy-2-nonenal (HNE). Therefore, the inhibition of ALR2 has received considerable attention as a means for the prevention and treatment of diabetic complications. To this purpose, a large number of ALR2 inhibitors (ARIs) have been developed in the past few decades (Figure 2)[6–9]. Unfortunately, few of these have advanced to the later stages of clinical trials. At present, epalrestat is only the drug available on the market for treatment of diabetic complications. Therefore, the development of more ARIs suitable for use to prevent DM complications is still needed.

It has been suggested that the discovery of ARIs possessing antioxidant activity is very desirable when designing drug candidates that are effective against diabetic complications, since increased oxidative stress is a widely accepted participant in the development and progression of DM and its complications[3,10–13]. A phenolic group is commonly involved in many natural and synthetic products exhibiting antioxidant activity[14–16]. Previously, we have designed a series of ARIs based on the scaffold of pyrido[2,3-e][1,2,4]thiadiazine-1,1-dioxide[17,18]. The present study is focused on the introduction of phenolic groups into the framework in order to obtain ARIs with antioxidant functionality.

Results and discussion

The key intermediate compounds **1** in the synthesis were obtained by starting from 2-amino-pyridines, and the procedure has been previously reported[17].

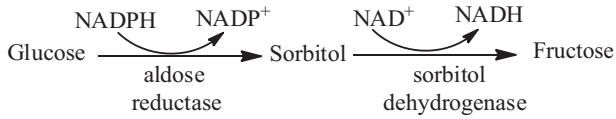

Figure 1. The polyol pathway of glucose metabolism.

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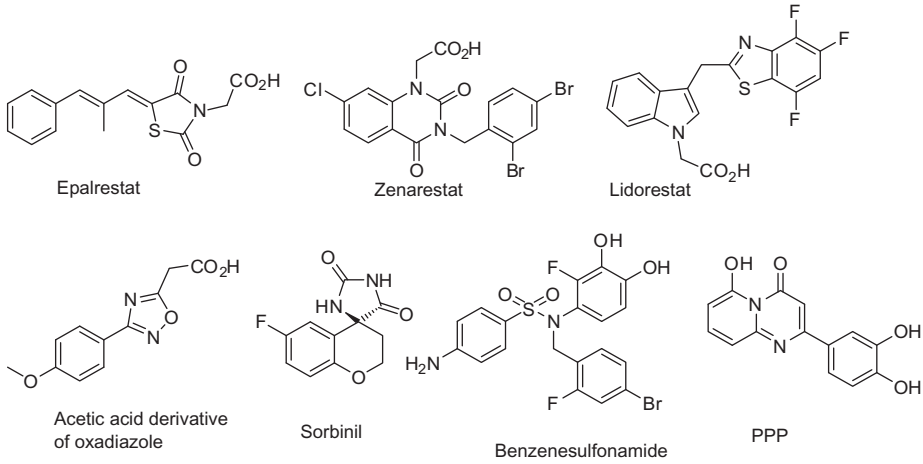
Figure 2. Previously reported aldose reductase inhibitors.



The introduction of a benzyl group as a side chain at the N2-position of esters **1a–c** was achieved by an alkylation reaction with benzyl bromide as appropriate to achieve the corresponding methyl esters **2a–c**, **4a–c** and **7**, which were then treated with different methods to form the final carboxylic acids[18–20]. The procedures are depicted in Schemes 1–3, respectively.

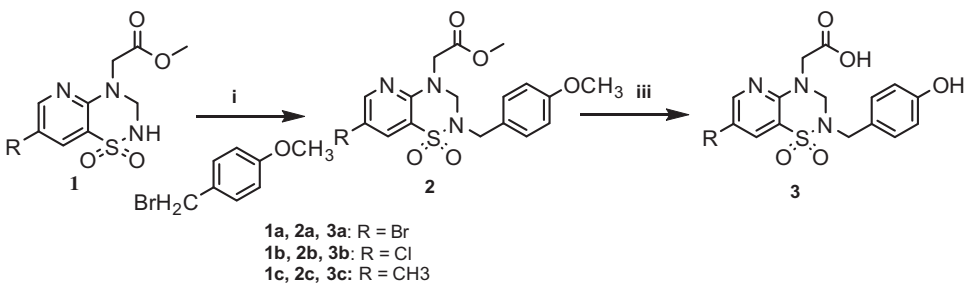
Compounds **2a–c** were demethylated to form the final acids **3a–c** by treatment with BBr_3.

The preparation of compounds **6a–c** proceeded from **4a–c**, which involved two steps as they were first converted into **5a–c** by hydrolysis with aqueous sodium hydroxide (Scheme 2). Compounds **11** and **12** were obtained by demethylation with $AlCl_3$ (Scheme 3).

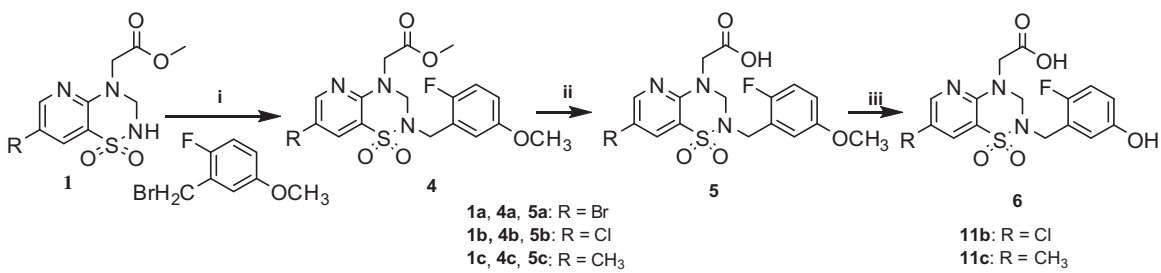
The synthesized compounds were evaluated for their ALR2 inhibition, scavenging effects on the DPPH free radical, and MDA inhibition. The results are summarized in Table 1. Previously reported compounds[17] were also included and named as **13a–c** in Table 1 for comparison.

ALR2 inhibitory activity

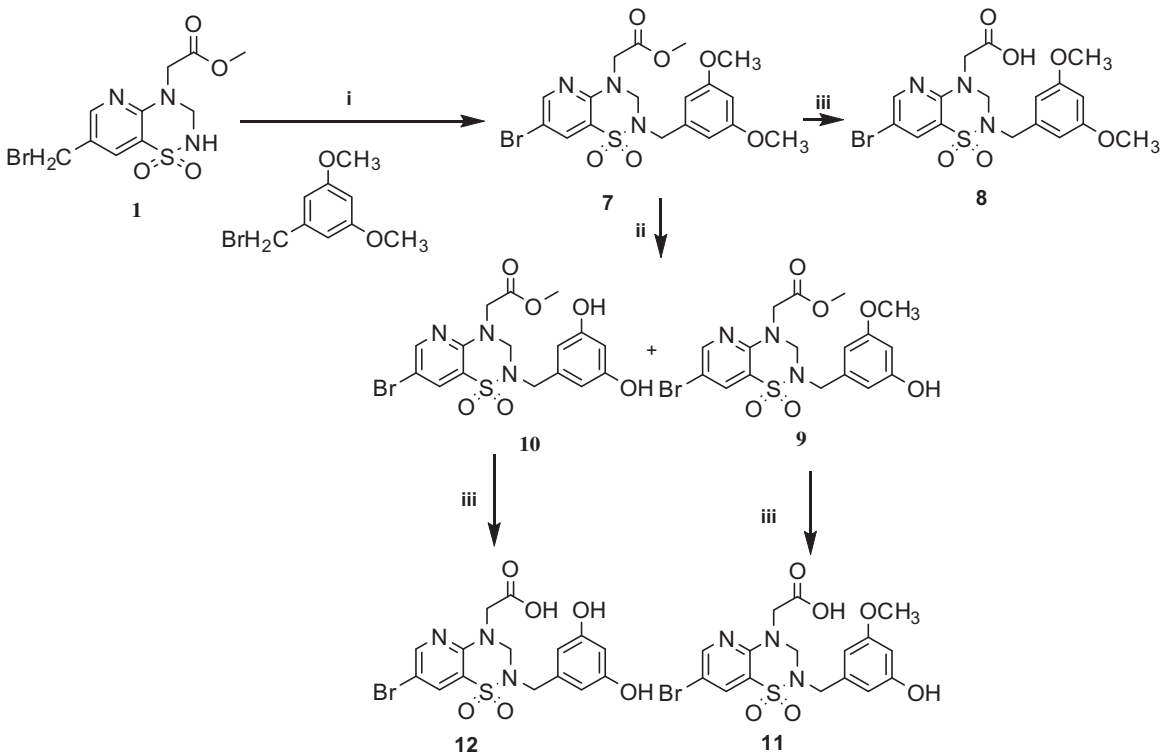
The synthetic work led to a series of compounds containing diversified groups located on the C7-position of the core and the N2-benzyl ring as shown in Table 1. All the compounds could be categorized according to substituents at the N2-benzyl ring in two types: the first type included **5a–c** and **6b–c**, which contained both an electron-donating group and fluoro, an electron-withdrawing group, at the N2-benzyl ring. The second type included those with only the electron-donating group at the N2-benzyl ring, namely compounds **13a–c**, **8**, **11** and **12**. Of all the compounds, **5a–c** and **6b–c** in the first type showed a more potent inhibitory activity against ALR2 with IC_{50} values ranging from 1.3 to 2.86 μM. The compounds in the second type bearing only electron-donating substituents at the N2-benzyl ring, namely **13b–c**, **3a–c**, **8**, **11** and **12**, displayed less inhibitory activity against ALR2. These results suggest that electron-donating substituents at the N2-benzyl ring retained ALR2 inhibition activity, and the electron-withdrawing substituents at the N2-benzyl ring improved the activity of ARIs based on the pyrido[2,3-e]-[1,2,4]-thiadiazine 1,1-dioxide scaffold. This is consistent with a previous study[17]. In addition, in comparing the series **3a–c** with the parent compounds **13a–c**, an interesting inhibition profile was observed. The C7-halogen, N2-4-hydroxyl derivatives **3a** and **3b** showed a higher activity than **13a** and **13b**, respectively. In contrast, the C7- methyl, N2-4-hydroxyl derivatives **3c** appeared to be less active than **13c**. These findings



Scheme 1. Reagents and conditions: (i) K_2CO_3, (ii) CH_3CN, 70 °C; (iii) BBr_3, CH_2Cl_2, 0 °C.

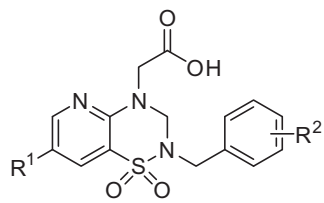


Scheme 2. Reagents and conditions: (i) K_2CO_3, CH_3CN, 70 °C; (ii) NaOHaq., THF, then HClaq. and (iii) K_2CO_3, thiophenol, NMP, 150 °C.



Scheme 3. Reagents and conditions: (i) K_2CO_3, CH_3CN, 70 °C; (ii) $AlCl_3$, CH_2Cl_2, 40 °C and (iii) NaOHaq., THF, then HCaq.

Table 1. Biological evaluation of pyridothiadiazine derivatives.



	Product						
Comp. no.	R^1	R^2	ALR2 IC_{50} (μM)*	DPPH inhibition, *n* (%)†	MDA (nmol/mgpro)‡	MDA inhibition, *n* (%)¶	MDA IC_{50} (μM)§
13a	Br	4-OCH_3	18.2 (0.52–20.76)	6.2	1.115 ± 0.008	2.6	–
13b	Cl	4-OCH_3	7.54 (4.32–8.01)	5.9	1.125 ± 0.018	1.6	–
13c	CH_3	4-OCH_3	7.2 (4.79–7.98)	3.1	1.056 ± 0.017	8.4	–
3a	Br	4-OH	10.1 (7.85–13.92)	17.0	0.838 ± 0.001	29.9	–
3b	Cl	4-OH	6.4 (3.21–10.13)	93.2	0.392 ± 0.156	73.9	124.4 (74.7–153.5)
3c	CH_3	4-OH	12.4 (4.85–16.54)	5.2	1.152 ± 0.034	0	–
5a	Br	2-F,5-OCH_3	1.3 (1.03–1.24)	8.7	1.115 ± 0.004	2.3	–
5b	Cl	2-F,5-OCH_3	1.74 (0.79–1.75)	8.6	1.012 ± 0.022	12.7	–
5c	CH_3	2-F,5-OCH_3	1.82 (0.71–2.12)	6.4	1.143 ± 0.021	0	–
6b	Cl	2-F,5-OH	2.86 (1.31–3.16)	17.2	1.036 ± 0.009	10.4	–
6c	CH_3	2-F,5-OH	2.86 (1.58–4.31)	9.8	1.138 ± 0.053	0.3	–
8	Br	3-OCH_3,5-OCH_3	14.2 (4.10–17.91)	11.5	1.109 ± 0.010	3.2	–
11	Br	3-OH,5- OCH_3	11.7 (6.22–14.38)	21.3	0.934 ± 007	20.4	–
12	Br	3-OH,5-OH	13.1 (5.77–12.07)	49.8	0.799 ± 0.005	38.7	–
Epalrestat			0.12 (0.08–0.13)	0	1.148 ± 0.032	0	–
Trolox				95.0	0.052 ± 0.002	98.1	–
Blank					0.127 ± 0.002		
Control					1.141 ± 0.056		

*IC_{50} (95% CL) values represent the concentration of the tested compounds required to decrease enzymatic activity by 50%.

†Inhibitory effects were evaluated at a concentration of 100 μM.

‡Blank: brain homogenate; control: brain homogenate + the oxidant system ($FeCl_3$ 20 μM and ascorbic acid 100 μM); the inhibitory effect was estimated at a concentration of 100 μM.

¶The MDA inhibitory activity % was calculated using the following equation: Inhibitory effect % = [(control − test)/(control − blank)] × 100%. Blank: brain homogenate; control: brain homogenate + the oxidant system ($FeCl_3$ 20 μM and ascorbic acid 100 μM); test: brain homogenate + the oxidant system ($FeCl_3$ 20 μM and ascorbic acid 100 μM) + compound.

§IC_{50} (95% CL) values represent the concentration of the tested compounds required to inhibit the production of MDA by 50%.

indicated that the introduction of a phenolic group does not necessarily increase inhibitory activity against ALR2. This is further evidenced by the fact that compound **11** is more active than compound **8** but less active than compound **12**, resulting in the rank order **11** >**12** >**8**. In the case of compounds **13b** and **3b**, the introduction of a hydroxyl group increased the activity, which is likely due to the enhanced binding behavior between **3b** and ALR2.

DPPH radical scavenging activity

The free radical scavenging potential of all the compounds were assessed *in vitro* using a model reaction with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Results are summarized in Table 1. While their free radical scavenging activity was less than that of Trolox, the positive control for antioxidant potency, compounds **3a**,**b**, **6b**, **8**, **11** and **12** showed good to excellent DPPH radical scavenging activity, and compound **3b** was the most active achieving 93.2% activity at a concentration of 100 μM, which approached that of Trolox (95%).

Structure-activity relationship studies were based on three comparisons. Among all the compounds in Table 1, the compounds containing a phenolic hydroxyl group showed higher free radical scavenging activity compared to their parent analogs, revealing that the phenolic subunit is a structural determinant of DPPH free radical scavenging activity in the present study. This is further confirmed by the effect of the number of the hydroxyl group in compounds **8**, **11**, **12** on the DPPH radical scavenging activity in which the hydroxyl group-containing products **11**, **12** formed from **9**, and **10**, respectively showed a much greater free radical scavenging activity (21.3 and 49.8%, respectively) compared with their parent compound **8** (11.5%), and compound **12** containing two hydroxyl groups showed more than twice the activity of compound **11** containing one hydroxyl group. Of all the hydroxyl contained compounds, with respect to the effect of a C7-substituent at the core only, the C7-chloro substitution undoubtedly had a more remarkable influence on activity than that of C7-bromo and C7-methyl, when comparing **3b** with **3a** and **3c**, and **6b** with **6c**.

Inhibition of lipid peroxidation

To further confirm the antioxidant potential of the newly synthesized compounds described above, the inhibitory effect of the compounds on hydroxyl radical dependent lipoperoxidation induced in rat brain homogenate by the oxidant system Fe(III)/ascorbic acid was also examined. The level of MDA is an indicator of lipid peroxidation in cellular membranes. In membranes, relative antioxidant reactivity is probably different from that in a homogeneous system, because of the role played by additional factors such as the subcellular location of the antioxidants and radicals, and to some extent, the differing activities between aqueous and lipophilic compartments. The antioxidation results from the DPPH radical scavenging experiment were perfectly replicated in this assessment demonstrating suppression of lipid peroxidation with the compounds. As shown in Table 1, the tested compounds inhibited the production of MDA and the thiobarbituric acid reactive substances (TBARS), and reduced the

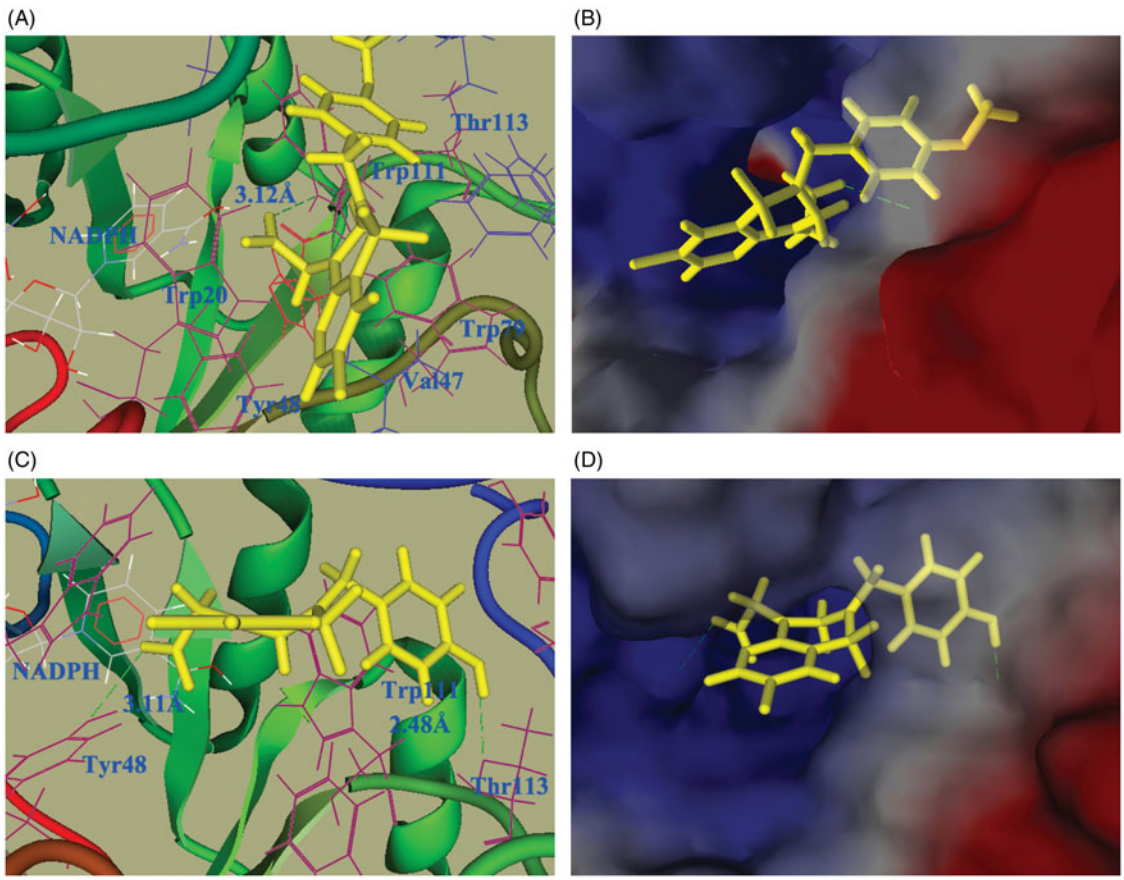


Figure 3. Molecular docking of compound **13b** (A & B) and **3b** (C & D). (A & C) Docking of compounds **13b** or **3b** into the ALR2 active site. The protein structure is shown in ribbon and tube representation with selected residues labeled and shown in line representation; ligand **13b** (or **3b**) and NADPH are shown as stick models. The docked pose of **13b** and **3b** are shown in yellow. Hydrogen bonds are shown as green dashed lines. (B & D) Surface representation of protein active site residues in the docking of **13b** or **3b** into the active site of ALR2.

index of lipid peroxidation, with varying degrees of efficacy. Compounds **3a**,**b**, **10b**, **11b**, **11** and **12** showed appreciable antioxidant properties, and were more potent than the other tested compounds. In particular, the inhibitory activity of the most active compound **3b** was 73.9% with an IC_{50} value of 124.4 μM, further demonstrating the importance of the hydroxyl substituents in the derivatives for their antioxidant activity. This is consistent with the results that were determined by the DPPH method. Antioxidants are known to act at different levels in the cellular oxidative pathway, showing multiple mechanisms of action. The degree of efficacy in inhibiting the level of MDA indicated the capacity of the compound to scavenge free radicals, or prevent the formation of free radicals, or both.

Molecular docking

To understand the mechanistic details in the inhibition of ALR2 and the importance of the hydroxyl group on the N2-benzyl ring, molecular docking of compounds **13b** and **3b**, a pair of structural analogs with **3b** possessing good activities both in ALR2 inhibition and antioxidant activity, were performed on a human ALR2 complex with $NADP^+$ and IDD594 (PDB code = 1US0). Docking results showed that compounds **13b** and **3b** bound well to the active site of ALR2 (Figure 3A and C). The carbonyl group of **13b** was inserted deeply into the anion binding site by a hydrogen bond interacting with the carbonyl oxygen atom and the side chains of residues Trp111 (3.12 Å). However, in the case of **3b**, in addition to the hydrogen bond between the carboxylic oxygen atom and Tyr48 (3.11 Å), an extra hydrogen bond (2.48 Å) was formed between the oxygen atom of the hydroxyl group on the N-benzyl ring with the residues of Thr113 (Figure 3C). These hydrogen bonding interactions deeply embedded compound **3b** in the anion bonding pocket, resulting in higher binding activity compared to **13b**.

Conclusions

The developed pyridothiadiazine derivatives having the electron withdrawing group at the C7 position and a hydroxyl group on the N2-benzyl ring were active in ALR2 inhibition. The hydroxyl group on the N2-benzyl ring was more effective than a methoxy group for ARI activity. Furthermore, compounds **3a–b** and **10** showed appreciable antioxidant properties in the tests both of DPPH scavenging and MDA inhibition. In addition, the increase in the number of the hydroxyl group on the N2-benzyl ring significantly promoted the DPPH scavenging activity and MDA inhibition of compounds.

Declaration of interest

This work was supported by the National Natural Science Foundation of China (Grant No. 21272025), the Research Fund for the Doctoral Program of Higher Education of China (Grant No. 20111101110042) and the Science and Technology Commission of Beijing, China (Grant No. Z131100004013003).

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Supplementary material available online