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Pterin-7-carboxamides as a new class of aldose reductase inhibitors

Ryota Saito [a,b,*], Saori Suzuki [a], Kaname Sasaki [a]

[a] Department of Chemistry, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan
[b] Research Center for Materials with Integrated Properties, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

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ABSTRACT

Aldose reductase is related to the onset and progression of diabetic complications, such as neuropathy, retinopathy, angiopathy, and so on: therefore molecules that are capable of inhibiting the enzyme are potential drugs for treatment of diabetic complications. Epalrestat is the sole aldose reductase inhibitor that is clinically used, but still has some drawbacks. Thus, the development of new aldose reductase inhibitors is still desired. We have synthesized a series of new pterin-7-carboxamides, and evaluated their in vitro inhibitory activities against human aldose reductase. All newly synthesized compounds exhibited the inhibitory activity. Among them, **1a** having a glycine side chain exhibits the highest activity comparable to that of sorbinil, a highly active aldose reductase inhibitor. Molecular docking of **1a** on the active site of the enzyme indicated this compound interacts with amino acid residues that are specific to the enzyme and related to suppressing side effects. Based on these results, we proved perin-7-carboxamides to be a new class of aldose reductase inhibitors, and particularly compound **1a** was found to be a good candidate for further biological investigations as a drug for treatment of diabetic complications with fewer side effects.

Aldose reductase (ALR2 or AKR1B1) belongs to the aldo–keto reductase (AKR) enzyme superfamily, and is the first enzyme in the polyol pathway, which reduces glucose into sorbitol in the presence of NADPH as a reductant.[1,2] Because this enzyme is implicated in the onset of diabetic complications, ALR2 inhibitors (ARIs) have been thought to be effective in preventing diabetic complications, and thus various inhibitors, such as epalrestat, sorbinil (Fig. 1), and others, have so far been developed.[1,2] However, most of these have failed clinical trials because of unfavorable side effects, low efficacy, or toxicity.[2] Currently, only epalrestat (Kinedak®) has been launched in the Japanese market as a chemotherapeutic agent for treating diabetic peripheral neuropathy.[3] However, during the six years after its launch, 17 cases of unexpected severe liver dysfunction, for which a causal relationship to the drug cannot be ruled out, have been reported.[4,5] Thus, the development of new ALR2 inhibitors is still strongly desired.

The whole active site of ALR2 consists of three distinct pockets.[2] The most important one is the catalytic site, or an "anion binding pocket", which is made up of Tyr48, His110, and Trp111 side chains and commonly found in enzymes belonging to the AKR superfamily.[6] Adjacent to this, there is a pocket composed of the residues Cys298, Leu300, Cys303, and Trp111. These residues are not conserved in other AKRs, and therefore this pocket is often designated as "the specificity pocket." The third one is a hydrophobic pocket formed by the residues Trp20, Phe122, and Trp219.

Aldehyde reductase (ALR1 or AKR1A1), another member of the AKR superfamily, has 65% sequence homology with ALR2, as well as structural homology.[7,8] This enzyme is often found in the kidneys and detoxifies or metabolizes toxic aldehydes; it is not associated with the onset of diabetic complications in spite of its similarity to ALR2. Therefore, it is important for ARIs to specifically interact with amino acid residues characteristic of ALR2, because the selectivity towards ALR2 and not ALR1 plays a role in suppressing the side effects.[7,8] With respect to this, it is known that Leu300 in the specificity pocket of ALR2 is replaced by Pro in ALR1, while the anion binding pocket of ALR1 is composed of the same amino acid residues as those of ALR2. Thus, the specific interaction with Leu300 as well as that with the aromatic residues composing the specificity pocket in ALR2 is considered essential for inhibitors to accomplish the enzyme-selective inhibition.[9] In this context, we take notice of pterin-7-carboxamides, reported as ricin toxin A-chain (RTA) inhibitors, because the pterin ring in these compounds is capable of interacting with an aromatic residue through an aromatic-aromatic interaction as well as hydrogen bonding with the protein backbone and amino acid residues adjacent to the aromatic residue.[10] These features of the pterin ring motivated us to utilize this unit as a key structure interacting with both an aromatic

* Corresponding author.

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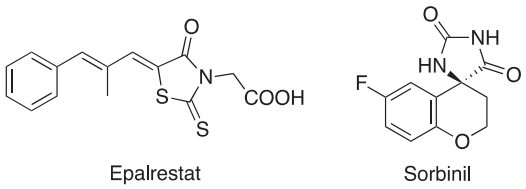


Figure 1. Structures of epalrestat and sorbinil.

residue and Leu300 in the specificity pocket of ALR2. In addition, peptide-conjugated pterin carboxamides were proven to be readily accessible,[11] indicating that the carboxylate functional group, which is required to attain high ALR2 inhibitory activity, can be readily introduced. However, no attempt to develop ARIs based on pterin-7-carboxamides has been made so far. Furthermore, pterin rings are found in some biologically profitable compounds, such as biopterin and folic acid (Fig. 2),[12] suggesting that newly designed ARIs synthesized from pterin are potential low-toxicity agents. Thus, in this study, several amino-acid-conjugated pterin-7-carboxamides have been synthesized and evaluated for their in vitro inhibitory activity against recombinant human ALR2 (h-ALR2) for the first time.

Synthesis of the pterin-7-carboxamides was achieved, as shown in Scheme 1, based on a previously reported method that utilizes DBU as a key additive to dissolve pterin in organic solvents and accelerate the reaction.[10,11] With this method, the pterin-amino acid conjugates (**1a**–**o**) were readily obtained by simply mixing pterin-7-carboxymethyl (7MCP) and unprotected amino acids in the presence of DBU in methanol.

The ALR2 inhibitory activity of the synthesized pterin-7-carboxamides (**1a**–**o**) was evaluated in vitro by measuring their inhibitory effects on the reduction of D,L-glyceraldehyde with recombinant h-ALR2 in the presence of coenzyme NADPH as a reductant.[13] When h-ALR2 reduces glyceraldehyde, coenzyme NADPH is oxidized to $NADP^+$. Therefore, the progress of the reduction reaction can be monitored spectrophotometrically by measuring the absorbance of NADPH at 340 nm. This method has been proven reliable.[14] The respective h-ALR2 inhibitory activities of **1a**–**o** were reported as their IC_{50} values, which express the 50% inhibition concentration of the compounds in the bioreduction. We also measured the inhibitory activity of pterincarboxylic acids (**2** and **3**) and folic acid for comparison with that of epalrestat used as a positive standard. All of the IC_{50} values are compiled in Table 1. It was found that **1a** exhibited the highest inhibitory activity (IC_{50} = 1.97 μM). This value was almost comparable to that of sorbinil, a highly active ARI ($IC_{50} \sim 2$ μM). Compounds **2**[15] and **3**,[16] which have a carboxy group directly attached to the pterin ring, yielded poor results. Compound **2** was found to be insoluble in the assay medium. Compound **3** showed some inhibition, with an IC_{50} = 26.5 μM, suggesting that a linker between the pterin ring and the carboxyl group is required. Folic acid exhibited weak but explicit inhibitory activity (IC_{50} = 52.5 μM). Owing to the synthetic difficulty, we have not constructed a library of 6-substitited pterin, but this result left the door open for further investigation of ALR2 inhibitory activity of 6-substituted pterins.

A careful examination of the observed h-ALR2 inhibitory data for **1a**–**o** reveals some structure–activity trends. First, compounds having L-amino acid residues showed markedly higher inhibitory activity than the D-counterparts (**1b**–**j**), indicating that pterins conjugated with L-amino acids are well suited for the polypeptide environment composed of L-amino acids.

Evaluation of the inhibitory activity of compounds having hydrophobic residues (**1b$_L$**–**e$_L$**, **1g$_L$**) showed that the activity apparently decreased with increasing size of the residues. To assess this observation quantitatively, the correlation between the pIC_{50} values and the physicochemical parameters presented in Table 1 was examined by means of the multiple linear regression analysis. Here, π represents the hydrophobicity parameter for the amino acid residues, ν is the upsilon steric parameter, and L, B_1, and B_5 are the STERIMOL length and maximum and minimum width parameters, respectively.[17] The following equation was obtained from the analysis as a statistically significant model:

$$pIC_{50} = 7.492 - 1.409B_1 - 0.232B_5 - 0.053L + 0.223\pi \quad (n = 10, r^2 = 0.951, s = 0.163) \tag{1}$$

Despite the limited number of samples (n = 10), the high coefficient of determination (r^2 = 0.951) and low standard error (s = 0.163) revealed that a good correlation was obtained. The Fischer test proved this equation to be significant with 99% confidence interval (p <0.01). It is obvious that the inhibitory activity can be explained predominantly with the steric parameters, B_1 and B_5. The negative sign of the coefficients for these parameters denotes the decrease in activity with an increase in the bulkiness of the amino acid side chain. A plot of the experimental versus predicted inhibitory activity using this model proved the predictive potential of this model (Fig. 3). When the IC_{50} values of **1f$_L$** and **1j$_L$** are compared with those of **1b$_L$** and **1g$_L$**, respectively, it was found that replacing one hydrogen in the residues of Ala and Phe with a hydroxy group, giving Ser and Tyr, respectively, markedly decreases the activity. Perhaps the hydroxy groups allow additional hydrogen bonding that negatively influences the fitting of the substrate to the active site.

We next considered the effect of alkyl chain length on the inhibitory activity. As can be seen from Table 1, it is obvious that there is no proportional relationship between the methylene chain length and activity. The activity decreased when the chain length increased from 1 to 3 and increased with elongation of the chain length until 5. The cause of this behavior is unclear, but it is likely that the flexible unbranched alkyl chains fold themselves to fit the whole structure appropriate to the enzyme's active site, especially when the chain length is 5. We also examined the pterin conjugated with Gly-Phe (**1o**), which has a chain length close to that of **1m**, and found it to be ineffective, probably because of the hydrogen-bondable amide functionality and the branched large benzyl group.

Overall, the simplest **1a** was the most potent ALR2 inhibitor among the compounds tested.

Next, in order to obtain a better insight into the interaction of the pterins with the active site, docking simulations were conducted. In these experiments, the protein structure coded as 2IKG[19] in the Protein Data Bank was employed because the struc-

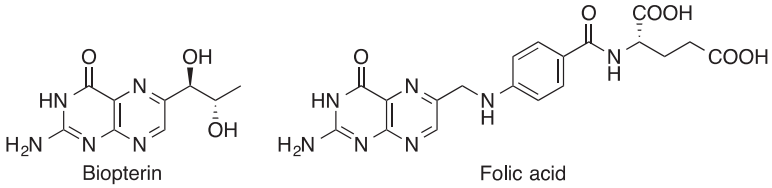
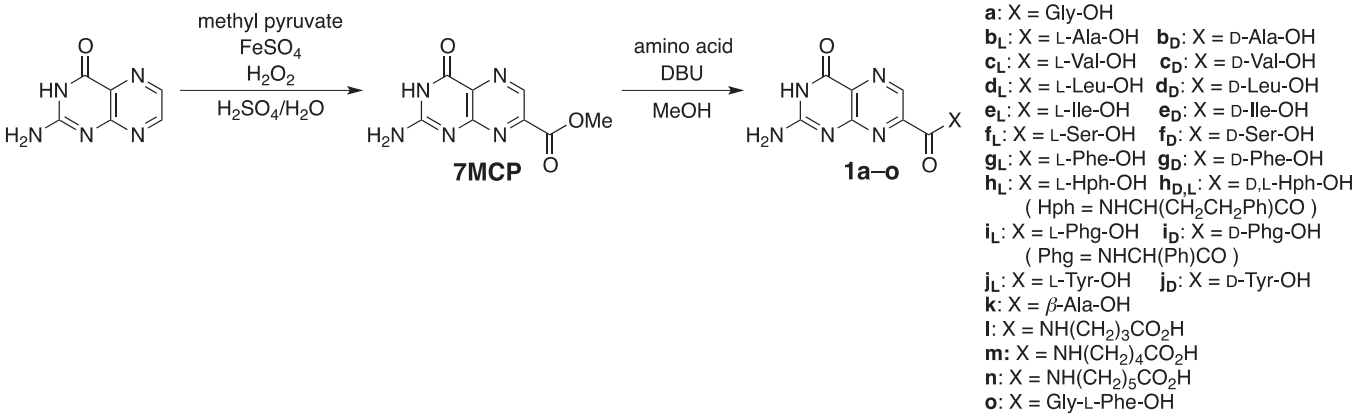
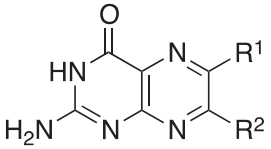


Figure 2. Structures of biopterin and folic acid.



Scheme 1. Synthesis of pterin-7-carboxamides **1a**–**o**.

Table 1
In vitro h-ALR2 inhibitory activity of pterin-7-carboxamides (**1a**–**o**), **2**, **3**, folic acid, and epalrestat, compiled with some physicochemical parameters

Compound	Entry	R^1	R^2	IC_{50} (μM)		π[a]	ν[b]	L[c]	B_1[d]	B_5[e]
				L-form	D-form					
	**1a**	H	CO-Gly-OH	1.97		0.00	0	2.06	1.00	1.00
	1b	H	CO-Ala-OH	12.4	72.0	0.31	0.52	2.87	1.52	2.04
	1c	H	CO-Val-OH	83.4	194	1.21	0.76	4.11	1.90	3.17
	1d	H	CO-Leu-OH	61.7	97.2	1.70	0.98	4.92	1.52	4.45
	1e	H	CO-Ile-OH	69.0	278	1.80	1.02	4.92	1.90	3.49
	1f	H	CO-Ser-OH	29.2	130	–0.04	0.53	3.97	1.52	2.70
	1g	H	CO-Phe-OH	46.0	68.1	1.79	0.70	4.62	1.52	6.02
	1h	H	CO-Hph[i]-OH	25.1	42.1[f]	2.10	0.70	8.33	1.52	3.58
	1i	H	CO-Phg[j]-OH	42.6	56.6	1.96	0.57	6.28	1.71	3.11
	1j	H	CO-Tyr-OH	216	580	0.96	0.70	4.73	1.52	6.72
	1k	H	CO–β-Ala-OH	17.5						
	1l	H	$CONH(CH_2)_3CO_2H$	25.5						
	1m	H	$CONH(CH_2)_4CO_2H$	11.7						
	1n	H	$CONH(CH_2)_5CO_2H$	4.02						
	1o	H	CO-Gly-Phe-OH	9% (10.5 mM)[g]						
	2	CO_2H	H	–[h]						
	3	H	CO_2H	26.5						
Folic acid				52.5						
Epalrestat				0.0665						

[a] Hydrophobicity parameters of amino acid residues.
[b] Upsilon steric parameters.
[c,d,e] STERIMOL length and maximum and minimum width, respectively.[17]
[f] D,L-form.
[g] % inhibition at a given concentration.
[h] Not determined, owing to the poor water solubility of the substrate.
[i] Hph stands for NHCH(CH_2CH_2Ph)CO (homophenylalanine).[18]
[j] Phg stands for NHCH(Ph)CO (phenylglycine).[18]

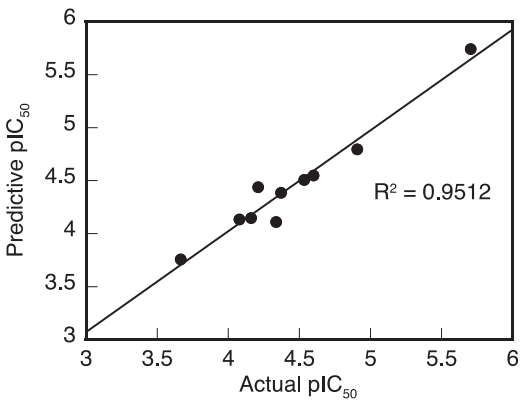


Figure 3. Plots of predictive pIC_{50} values for the model described by Eq. (1) versus the actual pIC_{50} values.

tural features of our pterins resemble the original ligand of 2IKG. All the docking experiments were carried out with the GOLD Suite software package.[20,21] ChemPLP scoring function was used for docking pose prediction, and the obtained poses were rescored with GOLDscore.[22] The best pose for each ligand was determined by considering the results obtained with both of these scoring functions.

First, we performed the docking simulation for **1a**. Figure 4 shows the predicted pose of **1a** in 2IKG structure. Obviously, **1a** tightly binds to the active site of ALR2 through hydrogen-bonding networks. The carboxylic acid moiety was well inserted in the anion-binding pocket to hydrogen bond with His110 and Tyr48. It should be noted that a water molecule assists the interaction of the amide NH of the inhibitor with Trp111. The pterin ring makes a π–π interaction with Trp111 and a hydrogen bond with Thr113 in the specificity pocket. The most noteworthy thing is that the carbonyl oxygen of the pterin makes a hydrogen bond with Leu300, which is significant for selective inhibition toward ALR2.

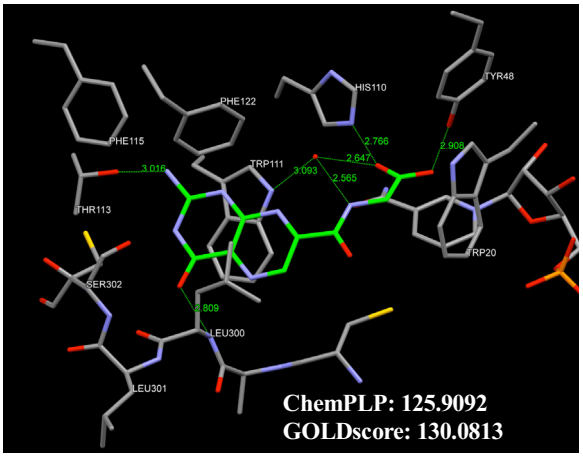


Figure 4. Docking of **1a** into the active site of h-ALR2 complied with the docking scores.

The predicted docking poses for $\mathbf{1b_L}$ and $\mathbf{1b_D}$ are shown in Figure 5 (a) and (b). It can be seen from these figures that the carboxylic acid moiety of $\mathbf{1b_L}$ makes a hydrogen bond with Tyr48, while $\mathbf{1b_D}$ does not. In addition, $\mathbf{1b_L}$ had higher docking score than $\mathbf{1b_D}$. Conceivably, this explains why compounds that have L-amino acid residues have higher inhibitory activity than the D-counterparts.

Figure 5(c) and (d) show the predicted docking poses for $\mathbf{1f_L}$ and $\mathbf{1f_D}$. As can be seen from these figures, hydroxy groups of the serine residues in both of the compounds form hydrogen bonds with His110 to misdirect the carboxylate group to the opposite side of the anion-binding site, while the pterin ring is accommodated in the specificity pocket by making the same interactions with Trp111, Thr113, and Leu300 as those observed for **1a**. This type of misdirection possibly comes about with $\mathbf{1j_L}$, which has a phenolic hydroxy group, and is likely to give rise to decrease the activity of these inhibitors.

In conclusion, we explored the synthesis and h-ALR2 inhibitory activity of pterin-7-carboxamides. From the results of the aldose reductase inhibitory activity evaluation, it was found that **1a** is the most effective inhibitor (IC_{50} = 1.97 μM). This value is nearly the same as that of sorbinil, a highly potent ARI ($IC_{50} \sim 2$ μM). From the docking study for **1a**, it was suggested that **1a** tightly interacts with amino acid residues composing the active site of ALR2. Notably, the specific interaction with Leu300 that is important for ALR2 selective inhibition was observed with **1a**. Thus, **1a** is a potential drug for treating diabetic complications that exhibit fewer side effects by only inhibiting the relevant h-ALR2.

Conflicts of interest

The authors declare no conflicts of interest.

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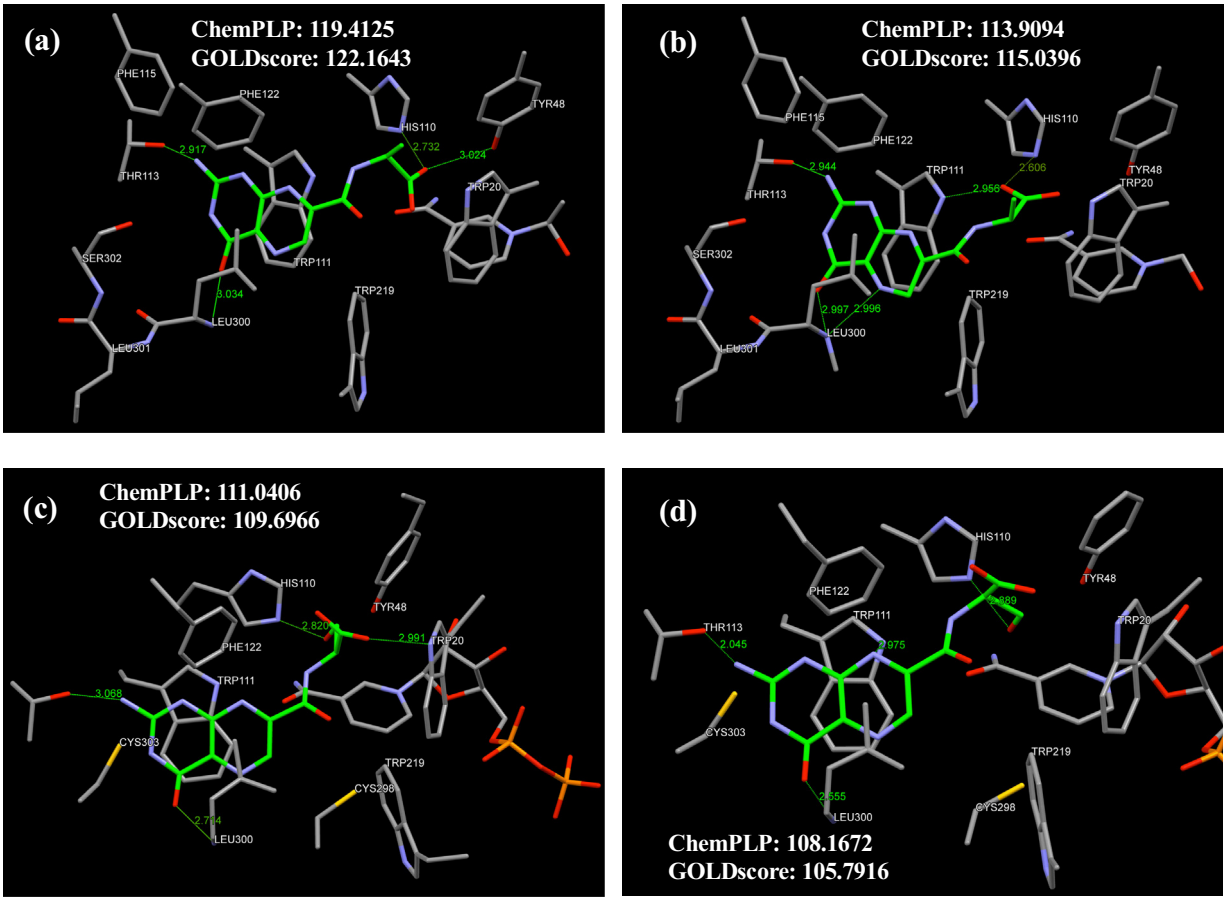

Figure 5. Docking poses for $\mathbf{1b_L}$ (a), $\mathbf{1b_D}$ (b), $\mathbf{1f_L}$ (c), and $\mathbf{1f_D}$ (d) in the active site of h-ALR2 complied with the docking scores.

Supplementary data

Supplementary data (experimental procedures, characterization, and in vitro methods) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.09.033. These data include MOL files and InChiKeys of the most important compounds described in this article.

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