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SHORT COMMUNICATION

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Novel 2-phenoxypyrido[3,2-*b*]pyrazin-3(4*H*)-one derivatives as potent and selective aldose reductase inhibitors with antioxidant activity

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ABSTRACT

To develop multifunctional aldose reductase (AKR1B1) inhibitors for anti-diabetic complications, a novel series of 2-phenoxypyrido[3,2-*b*]pyrazin-3(4*H*)-one derivatives were designed and synthesised. Most of the derivatives were found to be potent and selective against AKR1B1, and 2-(7-chloro-2-(3,5-dihydroxyphenoxy)-3-oxopyrido[3,2-*b*]pyrazin-4(3*H*)-yl) acetic acid (**4k**) was the most active with an IC$_{50}$ value of 0.023 μM. Moreover, it was encouraging to find that some derivatives showed strong antioxidant activity, and among them, the phenolic 3,5-dihydroxyl compound **4l** with 7-bromo in the core structure was proved to be the most potent, even comparable to that of the well-known antioxidant Trolox. Thus the results suggested success in the construction of potent and selective AKR1B1 inhibitors with antioxidant activity.

Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from defects in insulin secretion, insulin action, or both[1]. People suffering from DM are vulnerable to chronic diabetic complications, including neuropathy, retinopathy, nephropathy, and cataracts, which are the major menace to diabetic patients[2,3]. Increasing clinical research indicated that the abnormal polyol pathway flux of blood glucose is obviously related to pathogenesis of diabetes complications[4]. Normally, the blood glucose is predominantly converted to glucose-6-phosphate by hexokinase and then enters the glycolytic pathway. However, at high glucose concentration, specifically in diabetics, the combining capacity of aldose reductase (AKR1B1) to glucose is motivated and about one-third of the total glucose is metabolised through the polyol pathway in tissues such as lens, kidney, retina, and peripheral nerves[5–7]. In polyol pathway (Figure 1), AKR1B1 is the first enzyme and catalyses the reduction of glucose by NADPH to sorbitol, which can, in turn, be oxidised by the enzyme sorbitol dehydrogenase with concomitant reduction of NAD$^+$. In the tissues implicated in these pathologies, the increased polyol pathway flux would directly cause the accumulation of sorbitol, which is hard to penetrate through cellular membranes, resulting in osmotic imbalance, cell swelling, and membrane permeability changes. All these AKR1B1-mediated biochemical alterations contribute to the pathogenesis of diabetic complications[8–10]. Therefore, design and synthesis aldose reductase inhibitors (ARIs) to inhibit the activity of AKR1B1 and further regulate the polyol pathway of glucose are likely to be a promising therapy to prevent the development of diabetic complications.

Numerous variously ARIs (Figure 2) have been developed and some of them are endowed with excellent inhibitory activity[11–15]. However, most of the ARIs have 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 the accumulation of sorbitol is not enough to prevent and treat pathological changes in all the tissues. As the reduction of glucose consumes NADPH, which is required for regenerating reduced glutathione (GSH), this could induce intracellular oxidative stress (Figure 1). In addition, the abnormal decrease of NAD$^+$ gives rise to changes in cellular redox potentials and the activity of enzymes such as nitric oxide synthase (NOS) and GSH reductase would further exacerbate intracellular oxidative stress[16–18]. The pathogenesis of diabetic complications and hyperglycaemia-induced oxidative stress always promote to each other. Thus, it will be particularly important to develop ARIs having antioxidant activity, which keeps the enzyme in its reduced form and decreases the damage due to oxidative stress, and this could enhance the overall efficacy of ARIs targeted at diabetic complications.

Based on our previous work of structurally different ARIs[19–22], we have engaged in preparing a novel series of 2-phenoxypyrido[3,2-*b*]pyrazin-3(4*H*)-one derivatives as the new multifunctional ARIs with combined AKR1B1 inhibition and antioxidant activity. This study will focus on the further optimisation of the C2 side chain and the introduction of halogen substituent to the C7 position of the core structure.

Chemistry

The designed compounds with an acetic acid substituent at N4 position and a variety of phenoxyl substituents at C2 position of the pyrido[2,3-*b*]pyrazin-3(4*H*)-one scaffold were obtained by the syntheses starting from compounds **1a–c**, which was prepared
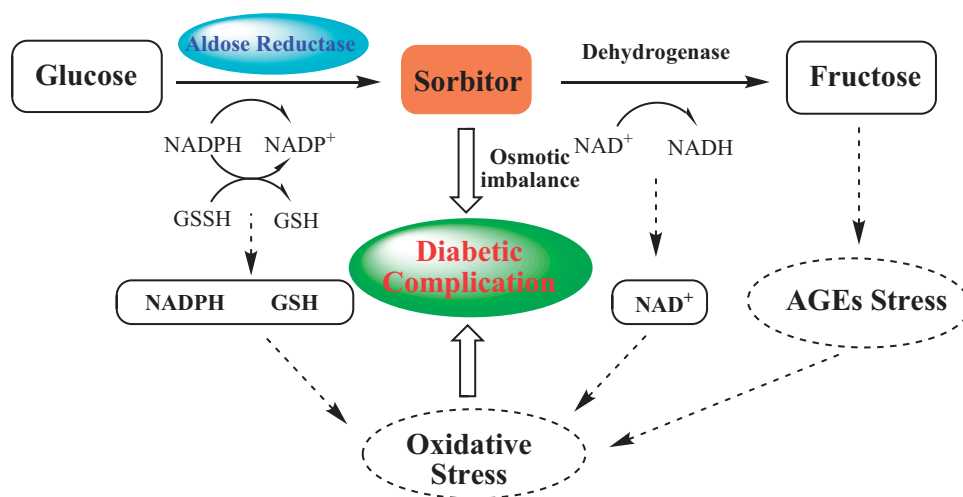
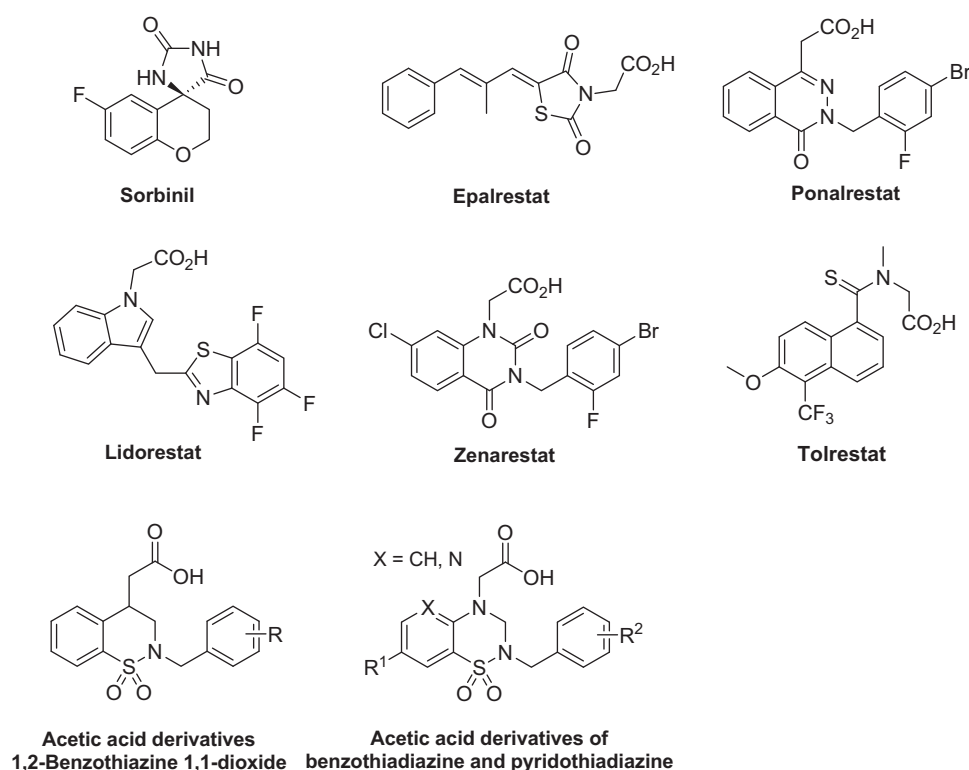**Figure 1.** Polyol pathway and pathogenesis of diabetic complications.

Figure 2. Structures of ARIs.

according to our previous reported methodology[20,21]. As shown in Scheme 1, compounds **1a–c** were firstly reacted with different phenolate to obtain compounds **2a–g**. Then the phenol methyl ethers **2a–g** were alkylated with methyl bromoacetate at the N4 position to form **3a–g** as key intermediates. Direct hydrolysis of **3a–e** with lithium hydroxide yielded compounds **4a–e**, and demethylation of partial or all the methoxyl with $AlCl_3$ followed by hydrolysis of **3b–g** gave the target compounds **4f–o**. The detailed experimental data was depicted in the supplemental material.

Results and discussion

All new synthetic compounds **4a–o** were tested for their potential inhibitory activity of AKR1B1 isolated from rat lenses. Besides, the inhibitory activity of aldehyde reductase (AKR1A1) isolated from

rat kidneys was also investigated to evaluate the selectivity for AKR1B1. The enzyme AKR1A1 is closely related to AKR1B1, and plays an important role in physiological detoxification. The validity of the inhibition results was assessed with respect to epalrestat as a positive ARI. Additionally, the antioxidant activity was assessed by using the model reaction with the stable free radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the modified method, which was first employed by Blois[23].

Inhibition of enzymes

As shown in Table 1, most of the 2-phenoxypyrido[3,2-b]pyrazin-3(4H)-one derivatives showed significant AKR1B1 inhibition and selectivity. Of all the compounds, 2–(7-chloro-2–(3,5-dihydroxyphenoxy)-3-oxopyrido[3,2-b]pyrazin-4(3H)-yl)acetic acid (**4k**) was the
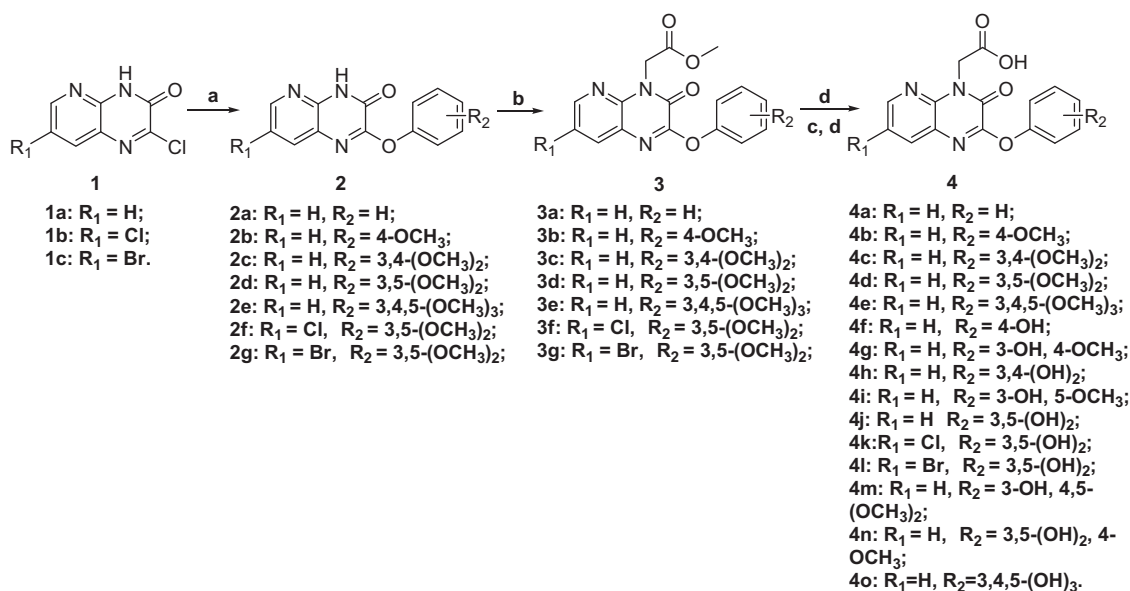
Scheme 1. a: PhOH, DMF, K_2CO_3, 75 °C; b: $BrCH_2COOCH_3$, K_2CO_3, CH_3CN, 55 °C; c: $AlCl_3$, CH_2Cl_2, 45 °C; d: $LiOH$, H_2O, THF, r.t., then 0.1 N HCl.

Table 1. Enzyme inhibition activity of 2–(3-oxo-2-phenoxypyrido[3,2-b]pyrazin-4(3H)- yl)acetic acid derivatives.

	Substituent		Inhibition/AKR1B1[a]	Inhibition/AKR1A1[a]
Compd.	R_1	R_2	$IC_{50}/\mu M$	$IC_{50}/\mu M$
4a	H	H	8.569 ± 0.523	26.914 ± 1.453
4b	H	4-OCH_3	2.684 ± 0.199	19.751 ± 1.323
4c	H	3,4-$(OCH_3)_2$	5.678 ± 0.608	17.623 ± 1.022
4d	H	3,5-$(OCH_3)_2$	4.297 ± 0.288	21.514 ± 1.807
4e	H	3,4,5-$(OCH_3)_3$	7.569 ± 0.431	20.874 ± 1.211
4f	H	4-OH	0.121 ± 0.010	15.942 ± 0.781
4g	H	3-OH, 4-OCH_3	2.500 ± 0.203	19.278 ± 1.812
4h	H	3,4-$(OH)_2$	0.305 ± 0.022	16.874 ± 0.692
4i	H	3-OH, 5-OCH_3	2.853 ± 0.031	15.142 ± 0.924
4j	H	3,5-$(OH)_2$	0.087 ± 0.007	20.812 ± 1.498
4k	Cl	3,5-$(OH)_2$	0.023 ± 0.003	18.357 ± 1.340
4l	Br	3,5-$(OH)_2$	0.056 ± 0.005	19.812 ± 1.407
4m	H	3-OH, 4,5-$(OCH_3)_2$	4.531 ± 0.258	31.557 ± 3.061
4n	H	3,5-$(OH)_2$, 4-OCH_3	2.357 ± 0.184	25.786 ± 1.650
4o	H	3,4,5-$(OH)_3$	0.859 ± 0.058	21.668 ± 1.473
Epalrestat	–	–	0.089 ± 0.010	55.712 ± 3.287

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

most active having an IC_{50} value of 0.023 μM and was more potent than epalrestat. In contrast, compound **4a**, which has no structural modification on the core structure and C2 phenoxyl side chain, was the lowest effective with an IC_{50} value of 8.596 μM. It is encouraging to find that introduction of phenolic hydroxyl or methoxyl to the C2 phenoxyl ring of **4a** could enhance the inhibitory activity. Compounds **4b–e**, **4g**, **4i**, **4m,** and **4n** containing one or more methoxyl on the C2-phenoxyl side chain displayed relatively low AKR1B1 inhibition with IC_{50} values ranging from 2.357 to 7.569 μM. Further demethylation of all methoxyl group leading to compounds **4f**, **4h**, **4j,** and **4o** showed an obvious enhancement in AKR1B1 inhibition with IC_{50} values in the range between 0.087 and 0.859 μM. The phenolic hydroxyl in C2 phenoxyl ring had an effect on AKR1B1 inhibition with the rank order of 3,5-$(OH)_2$>4-OH > 3,4-$(OH)_2$>3,4,5-$(OH)_3$>3,5-$(OH)_2$, 4-OCH_3>3-OH, 4-OCH_3>3-OH, 5-OCH_3>3-OH, 4,5-$(OCH_3)_2$. Furthermore, the

halogen substituent (Cl or Br) was introduced at the C7 position of compound **4j** giving **4k** and **4l**, which largely increased the inhibitory activity. Analysis of the structure–activity relationship (SAR) indicated that introduction of both phenolic hydroxyl to the C2 phenoxyl ring and halogen substituent at the C7 position of the core enhanced the AKR1B1 inhibitory activity. In addition, all compounds were also tested for their inhibition ability against AKR1A1, and showed low activity with IC_{50} values more than 15 μM, demonstrating good selectivity for AKR1B1.
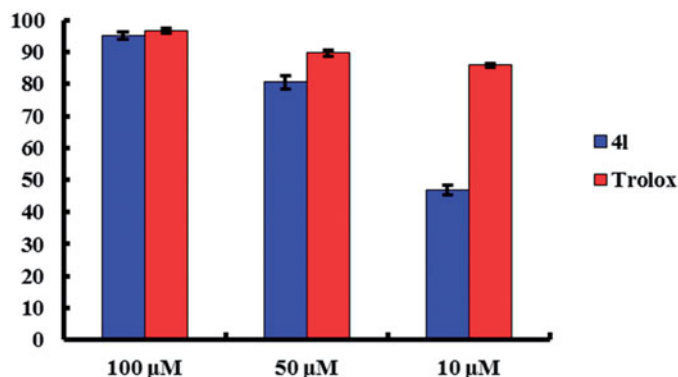
The antioxidant activity

The antioxidant activity of all synthesised compounds was determined with the DPPH model reaction and 6-hydroxy-2,5,7,8-chroman-2-carboxylic acid (Trolox) was employed as a positive control

(Table 2). Most of the derivatives containing phenolic hydroxyl showed good DPPH radical scavenging activity ranging from 21.5 to 80.6% at the concentration of 50 μM. Of all tested compounds, **4l** with 3,5-dihydroxyl on the C2 phenoxyl ring and 7-bromo on the core structure showed the best scavenging activity, that is, 95.3, 80.6, and 46.8% at concentrations of 100, 50, and 10 μM,

Table 2. Antioxidant activity of 2–(3-oxo-2-phenoxypyrido[3,2-*b*]pyrazin-4(3*H*)-yl)acetic acid derivatives.

Compd.	Substituent		DPPH/sca%		
	R₁	R₂	100 μM	50 μM	10 μM
4a	H	H	6.9 ± 1.2	–	–
4b	H	4-OCH₃	18.9 ± 0.8	–	–
4c	H	3,4-(OCH₃)₂	21.2 ± 1.5	–	–
4d	H	3,5-(OCH₃)₂	25.9 ± 1.3	–	–
4e	H	3,4,5-(OCH₃)₃	17.8 ± 0.9	–	–
4f	H	4-OH	37.7 ± 1.0	–	–
4g	H	3-OH, 4-OCH₃	54.8 ± 1.6	21.5 ± 1.2	–
4h	H	3,4-(OH)₂	74.6 ± 1.4	44.1 ± 2.3	–
4i	H	3-OH, 5-OCH₃	78.4 ± 0.7	45.2 ± 1.8	–
4j	H	3,5-(OH)₂	95.0 ± 0.9	77.2 ± 1.5	45.3 ± 1.9
4k	Cl	3,5-(OH)₂	94.8 ± 1.1	76.4 ± 0.9	49.1 ± 2.4
4l	Br	3,5-(OH)₂	95.3 ± 1.2	80.6 ± 2.0	46.8 ± 1.5
4m	H	3-OH, 4,5-(OCH₃)₂	56.9 ± 1.8	35.9 ± 1.1	–
4n	H	3,5-(OH)₂, 4-OCH₃	65.7 ± 0.9	40.8 ± 1.6	–
4o	H	3,4,5-(OH)₃	88.5 ± 1.4	65.2 ± 1.9	23.4 ± 2.0
Trolox		–	96.7 ± 0.8	89.8 ± 1.0	85.9 ± 0.6

**Figure 3.** Comparison on DPPH radical scavenging activity.
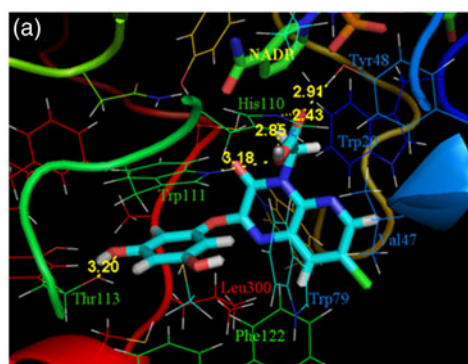
respectively, which had an almost similar activity compared with Trolox at high concentrations indicating the potency of antioxidant (Figure 3). SAR study of compounds **4b** *vs.* **4f**, **4c** *vs.* **4h**, **4d** *vs.* **4j**, and **4e** *vs.* **4o** indicated that the substitution of methoxyl with phenolic hydroxyl observably enhanced the radical scavenging activity. Regarding the position of phenolic hydroxyl on the C2 phenoxyl side chain, the 3,5-dihydroxyl substituent was the most effective in activity enhancement when comparing all the phenolic hydroxyl derivatives. However, comparison of compounds **4j**, **4k**, and **4l** revealed that the C7-halogen substituent had little impact on the radical scavenging activity.

Molecular docking

Compound **4k** endowed with excellent activities both in the AKR1B1 inhibition and antioxidant reaction was docked with the conformation of the human AKR1B1/NADP⁺/lidorestat complex (PDB code: 1Z3N), to understand the mechanistic details and the above-described SARs. As shown in Figure 4, compound **4k** fitted well to the active site of AKR1B1. The carboxylate group was inserted deeply in the anion binding site by forming tight hydrogen-bonding interactions with Tyr48 (2.91 Å), Trp111 (3.18 Å), and His110 (2.85 and 2.43 Å) and engaging in a stabilizing electrostatic interaction with the positively charged nicotinamide moiety of the NADP cofactor (N–O = 4.12 Å). Besides, the 3-hydroxyl oxygen atom of the C2 phenoxyl side chain formed an additional hydrogen bond with the side chain of Thr113 (3.20 Å), confirming the importance of phenolic hydroxyl on the activity enhancement of AKR1B1 inhibition. Moreover, the 3,5-dihydroxyphenoxyl ring of the C2 side chain was well placed into the specificity pocket and paralleled to the indole ring of Trp111 forming a stable stacking interaction. Meanwhile, the pyrido[2,3-*b*]pyrazine-3(4*H*)-one core structure matched very well the hydrophobic pocket and the 7-chloro substituent pointed towards the opening of the active cleft.

Conclusion

In conclusion, a series of novel ARI candidates (**4a–o**) based on pyrido[2,3-*b*]pyrazin-3(4*H*)-one core were synthesised and biologically evaluated for AKR1B1 inhibition and selectivity, as well as anti-oxidative properties through DPPH radical scavenging test. All compounds exhibited excellent AKR1B1 inhibitory activity in the IC₅₀ range of 0.023 to 8.569 μM. Therein, compounds **4j**, **4k,** and **4l** containing phenolic 3,5-dihydroxyl on the C2 phenoxyl side chain were not only sufficient to inhibit AKR1B1 but also effective
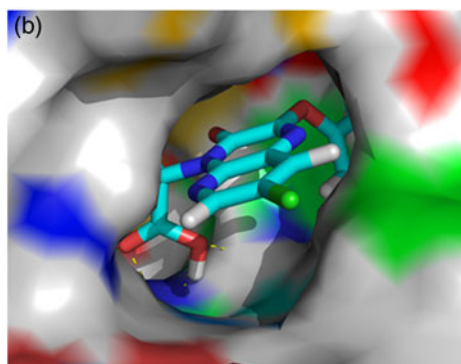
Figure 4. Docking of **4k** into the active site of AKR1B1. (a) The protein structure is shown in ribbon and tube representation with selected residues labeled and shown in line representation, ligand, and NADP are shown as stick models. The docked pose of **4k** is shown in cyan (C), red (O), blue (N), and green (Cl). Hydrogen bonds are shown as yellow dashed lines. (b) Protein residues are in surface representation.

for DPPH radical scavenging, which indicated success in the development of potent ARIs with antioxidant activity. Compound **4k** was the most potent against AKR1B1 and much less active against AKR1A1 suggesting distinguished selectivity, while compound **4l** showed the best DPPH scavenging activity even comparable with Trolox at high concentrations. The SAR studies indicated that the combination of phenolic 3,5-dihydroxyl in the C2-phenoxy group and C7-halogen (Cl or Br) in the core structure largely increased the AKR1B1 inhibitory activity, and the 3,5-dihydroxyl substituent contributed greatly to the activity enhancement of anti-oxidation when comparing all the phenolic hydroxyl derivatives, which provided a beneficial strategy for the discovery of new potent ARIs with antioxidant activity.

Disclosure statement

No potential conflict of interest was reported by the authors.

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