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Synthesis and biological evaluation of thiazolidine-2-thione derivatives as novel xanthine oxidase inhibitors

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Abstract

Xanthine oxidase (XO) is a key enzyme in the generation and development of hyperuricemia. Thiazolidine-2-thione, a typical heterocyclic compound, have been widely used in the field of drug synthesis. In this study, a series of novel thiazolidine-2-thione derivatives were synthesized as XO inhibitors, and the XO inhibitory potencies of obtained compounds were evaluated by in vitro enzyme catalysis. The result shown that compound 6k behaved the strongest XO inhibitory activity with an IC50 value of 3.56 μmol/L, which was approximately 2.5-fold more potent than allopurinol. The structure-activity relationship revealed that the phenyl-sulfonamide group was indispensable for thiazolidine-2-thione derivatives to produce XO inhibitory activity. The enzyme inhibition kinetics analyses confirmed that compound 6k exerted a mixed-type XO inhibition. Additionally, the molecular docking results suggested that the 4-fluorophenyl-sulfonyl moiety could interact with Gly260 and Ile264 in the innermost part of the active pocket through 2 hydrogen bonds, while the thiazolidine-thione moiety could form two hydrogen bonds with Glu263 and Ser347 in hydrophobic pockets. In summary, the results described above suggested that compound 6k could be a valuable lead compound for the treatment of hyperuricemia as a novel XO inhibitor.

1. Introduction

Xanthine oxidase (XO) is a key enzyme in purine catabolism in some species including humans, which plays a major role in the oxidation of hypoxanthine to xanthine, and then xanthine to uric acid (UA) [1, 2]. Overproduction of uric acid induces hyperuricemia due to abnormal XO activity, which is also linked with gout. Gout is a metabolic disease in which excessive levels of UA cause deposition of urate crystals in joints [3–5]. In addition, hyperuricemia is also associated with other diseases, such as inflammation [6], chronic kidney disease...
hypertensive disorders [9] and cardiovascular diseases [10]. XO inhibitors can block the biosynthesis of UA from purines, which can lower the production of UA. Allopurinol [11], febuxostat [9] and topiroxostat [12] are the clinical inhibitors of XO, used for the treatment of hyperuricemia. Current inhibitors of XO have several adverse effects (e.g. skin rashes, allergic reactions, increased blood pressure and increased risk of developing cataracts) [13–15], so there is a need of new XO inhibitors with better efficacy, and lower side effects.

Due to the structural characteristics and superior biological activity, heterocyclic compounds have a wide range of applications in the field of medicinal synthesis. Recently, research emphasis has switched to the discovery of novel effective and affordable XO inhibitors with minimal side effects based on heterocyclic compounds [16–18]. Thiazolines are the representatives of five-membered heterocyclic compound, which have been used in different areas of medicine [19, 20], materials [21], biological dyes [22], and ion receptors [23]. As a typical thiazolidine derivatives, thiazolidine-2-thione is an important organic intermediate in pharmaceuticals and agrochemicals. Thiazolidine-2-thione show diversified biological activity, such as aldose reductase inhibitors, anticancer, anti-inflammatory, and antifungal [24, 25]. At the same time, thiazolidine-2-thione has two tautomers [26, 27], including thione and thiol, which also has been widely used as chiral auxiliaries in catalytic asymmetric synthesis.

In this study, a series of novel thiazolidine-2-thione derivatives were designed and synthesized. In vitro XO inhibitory activity was researched using enzyme catalysis, and structure-activity relationships of thiazolidine-2-thione derivatives were described. The inhibitory mode of compound 6k was determined through enzyme inhibitory kinetics studies. In addition, molecular modeling study was also performed to investigate the inhibitory behaviors of compound 6k.

### 2. Materials and methods

#### 2.1 Chemistry synthesis

The chemicals for the synthetic reactions were purchased from Macklin Biochemical Co. Ltd, and other organic reagents were purchased from local reagent dealers. Melting points (m.p.) were obtained with MP-21 micro melting point apparatus. $^1$H and $^{13}$C NMR spectra were performed on a Bruker AV-400 nuclear magnetic resonance for solutions of the compounds in CDCl$_3$ at a temperature of 23–28°C, $J$ values are given in Hz. High-resolution mass spectra were recorded on an Agilent 6520 Series Q-TOF-MS system. All reactions were monitored by thin-layer chromatography (TLC).

**2.1.1 Preparation of 2-aminoethanol hydrogen sulfate (2).** Aminoethanol (9 mL, 0.15 mol) and H$_2$O (9 mL) were added 100 mL three-necked flask respectively at 0°C, and then, the mixture (16.3 mL, v/v = 1/1) of sulfuric acid (98%) and H$_2$O was slowly dropped into the flask. The reaction mixture was stirred at 0°C for 0.5 h. After the completion of the reaction, the mixture was added to absolute ethanol, filtered, and the filter cake was washed with absolute ethanol (3×15 mL). Drying under vacuum gave 2-aminoethanol hydrogen sulfate (19.2 g, yield 90.7%) and can be applied in next step without further purification.

2-aminoethanol hydrogen sulfate (2). white solid, m.p. 74–75°C. ESI-MS: m/z calc. for C$_2$H$_7$NO$_4$S 142.0175 [M+H]$^+$, found 142.0207 [M+H]$^+$.

**2.1.2 Preparation of thiazolidine-2-thione (3).** Intermediate 2 (7.06 g, 0.05 mol), KOH (5.61 g, 0.10 mol) and ethanol (100 mL) were added 100 mL three-necked flask respectively. The reaction mixture was heated to 40°C and carbon disulfide (7.61 g, 0.10 mol) were added in ten batches about 1 hour. The reaction mixture was further stirred at that temperature for 3 h. After the completion of the reaction, the mixture was allowed to cool to 5–10°C and washed with 5% sodium hydroxide solution (100 mL). The extract was washed with saturated brine...
(3×10 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure to obtain the crude. Dissolve the crude in absolute ethanol for recrystallization to obtain pure thiazolidine-2-thione (4.05 g, yield 68.1%).

**thiazolidine-2-thione (3).** A white solid, m.p.: 105–107°C, ESI-MS: m/z calc. for C₃H₅NS₂ 119.9942 [M+H]+, found 119.9913 [M+H]+.

### 2.1.3 General procedure for the preparation of compound 4a–4d.

Thiazolidine-2-thione (1.20 g, 10.0 mmol), NaOH (0.44 g, 11.0 mmol), and ethanol (40 mL) were added 100 mL three-necked flask respectively at 40°C. After NaOH was completely dissolved, a mixed solution of bromoethane/bromopropane (15.0 mmol) and ethanol (15 mL) was slowly added dropwise using a constant pressure funnel. Then the temperature was raised to 50°C and the reaction was carried out for 5 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the mixture was allowed to cool to room temperature and extracted with ethyl acetate (3×10 mL). The extract was washed with saturated brine (3×10 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography eluting with a mixture of petroleum ether and ethyl acetate (10:1–1:1).

- **3-ethyl-thiazolidine-2-thione (4a).** Colorless oily liquid (1.06 g, yield 71.9%). ¹H-NMR δH (400 MHz, CDCl₃): 4.21 (t, J = 7.8 Hz, 2H), 3.38 (q, 2H), 3.10 (t, J = 7.2 Hz, 3H). ¹³C-NMR δC (100 MHz, CDCl₃): 163.44, 64.37, 35.23, 27.09, 14.69. ESI-MS: m/z calc. for C₆H₁₃NS₂ 148.0855 [M+H]+, found 148.0801 [M+H]+.

- **3-propyl-thiazolidine-2-thione (4b).** Colorless oily liquid (1.01 g, yield 68.3%). ¹H-NMR δH (400 MHz, CDCl₃): 4.07 (t, J = 6.0 Hz, 2H), 3.75 (t, J = 6.0 Hz, 2H), 3.27 (t, J = 9.0 Hz, 2H), 1.65 (m, 2H), 0.87 (t, J = 6.0 Hz, 3H). ¹³C-NMR δC (100 MHz, CDCl₃): 164.75, 67.14, 59.62, 38.31, 27.22, 14.58. ESI-MS: m/z calc. for C₉H₁₇NS₂ 162.0412 [M+H]+, found 162.0433 [M+H]+.

- Thiazolidine-2-thione (1.20 g, 10.0 mmol), NaOH (0.44 g, 11.0 mmol), CuI (0.10 g, 0.5 mmol) and ethanol (40 mL) were added 100 mL three-necked flask respectively at 60°C. After NaOH was completely dissolved, a mixed solution of bromoethane/bromopropane (15.0 mmol) and ethanol (15 mL) was slowly added dropwise using a constant pressure funnel. Then the temperature was raised to 80°C and the reaction was carried out for 16 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the mixture was allowed to cool to room temperature and extracted with ethyl acetate (3×10 mL). The extract was washed with saturated brine (3×10 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography eluting with a mixture of petroleum ether and ethyl acetate (10:1–1:1).

- **3-phenyl-thiazolidine-2-thione (4c).** Colorless oily liquid. (1.28 g, yield 66.7%). ¹H-NMR δH (100 MHz, CDCl₃): 7.72 (d, J = 13.1 Hz, 2H), 7.33 (t, J = 10.4 Hz, 2H), 7.09 (t, J = 8.3 Hz, 2H), 3.81 (t, J = 5.4 Hz, 2H), 3.27 (t, J = 14.3 Hz, 2H). ¹³C-NMR δC (100 MHz, CDCl₃): 191.34, 152.27, 137.51, 130.44, 128.83, 44.19, 35.72. ESI-MS: m/z calc. for C₁₀H₉NS₂ 196.0255 [M+H]+, found 196.0207 [M+H]+.

- **3-benzyl-thiazolidine-2-thione (4d).** White powder solid. (1.44 g, yield 68.9%), mp: 115–117°C. ¹H-NMR δH (400MHz, CDCl₃): 7.34 (m, 5H), 4.42 (d, 2H), 4.25 (t, J = 9.0 Hz, 2H), 3.41 (t, J = 9.0 Hz, 2H). ¹³C-NMR δC (100 MHz, CDCl₃): 197.15, 138.82, 128.94, 126.52, 124.43, 57.27, 44.92, 23.61. ESI-MS: m/z calc. for C₁₀H₁₁NS₂ 210.0412 [M+H]+, found 210.0405 [M+H]+.

### 2.1.4 Preparation of 2-thioxothiazolidine-3-carbonyl chloride (5).

Thiazolidine-2-thione (1.20 g, 10.0 mmol), triethylamine (TEA, 1.34 g, 13.2 mmol), and CH₂Cl₂ (80 mL) were added 250 mL three-necked flask respectively and stirred at 0°C. Triphosgene (1.32 g, 4.4 mmol) was dissolved in CH₂Cl₂ (20 mL) and slowly added dropwise to three-necked flask...
using a constant pressure funnel. Then, the reaction mixture was stirred overnight at room temperature. After completion of the reaction as indicated by TLC, the mixture was poured to pre-cooled 1mol/L HCl (60 mL), and the product was extracted with CH₂Cl₂ (3×10 mL). The combined organic phase was washed successively with saturated sodium bicarbonate and sodium chloride, dried over sodium sulfate, and concentrated in vacuo. The solid was recrystallized with petroleum ether to obtain yellow crystals 2-thioxothiazolidine-3-carbonyl chloride (1.46 g, yield 80.37%), m.p. 88–90°C.

2.1.5 General procedure for the preparation of compound 6a-6k. Amine (10 mmol), TEA (0.67g, 6.6 mmol) and tetrahydrofuran (THF, 40 mL) were added 100 mL three-necked flask respectively and stirred at 0°C. Compound 

N-ethyl-2-thioxothiazolidine-3-carboxamide (6a). Light yellow oil liquid (1.18 g, yield 90.08%). ¹H-NMR δ_H (400 MHz, CDCl₃): 9.89 (s, 1H), 4.76 (t, J = 6.0 Hz, 2H), 3.99 (t, J = 9.0 Hz, 2H), 3.26 (t, J = 7.8 Hz, 2H), 1.15 (t, J = 5.4 Hz, 3H). ¹³C-NMR δ_C (100 MHz, DCl₃): 200.22, 151.36, 56.27, 44.61, 27.13. ESI-MS: m/z calc. for C₆H₁₀NO₂S₂ 213.0235 [M+Na]⁺, found 213.0234 [M+Na]⁺.

N-propyl-2-thioxothiazolidine-3-carboxamide (6b). Light yellow oil liquid (0.94 g, yield 76.68%). ¹H-NMR δ_H (400 MHz, CDCl₃): 9.80 (s, 1H), 4.78 (t, J = 6.0 Hz, 2H), 3.31–3.25 (m, 4H), 1.54–1.66 (m, 2H), 0.96 (t, J = 6.0 Hz, 3H). ¹³C-NMR δ_C (100 MHz, DCl₃): 200.16, 152.32, 56.35, 42.40, 27.10, 22.42, 11.55. ESI-MS: m/z calc. for C₇H₁₂NO₂S₂ 227.0283 [M+Na]⁺, found 227.0282 [M+Na]⁺.

N-butyl-2-thioxothiazolidine-3-carboxamide (6c). Light yellow oil liquid (1.02 g, yield 77.86%). ¹H-NMR δ_H (400 MHz, CDCl₃): 9.97 (s, 1H), 4.89 (m, 2H), 3.82 (m, 2H), 3.53 (m, 2H), 1.66 (m, 2H), 1.23 (t, J = 6.0 Hz, 2H). ¹³C-NMR δ_C (100 MHz, DCl₃): 200.16, 152.32, 56.30, 40.38, 31.15, 27.06, 20.12, 13.65. ESI-MS: m/z calc. for C₈H₁₄NO₂S₂ 241.0439 [M+Na]⁺, found 241.0420 [M+Na]⁺.

N-isobutyl-2-thioxothiazolidine-3-carboxamide (6d). Light yellow oil liquid (0.92 g, yield 70.23%). ¹H-NMR δ_H (400 MHz, CDCl₃): 9.84 (s, 1H), 4.70 (t, J = 9.0 Hz, 2H), 3.28 (t, J = 7.8 Hz, 2H), 3.16 (t, J = 5.7 Hz, 2H), 1.86 (m, 1H), 0.95 (d, J = 6.0 Hz, 6H). ¹³C-NMR δ_C (100 MHz, DCl₃): 200.32, 152.43, 56.36, 48.19, 28.13, 27.09, 20.22. ESI-MS: m/z calc. for C₉H₁₄NO₂S₂ 261.0439 [M+Na]⁺, found 261.0429 [M+Na]⁺.

N-phenyl-2-thioxothiazolidine-3-carboxamide (6e). Yellow solid powder (1.10 g, 6.0 mmol) was dissolved in THF (10 mL) and slowly added dropwise to three-necked flask using a constant pressure funnel. Then, the reaction mixture was stirred for 8 h at room temperature and monitored by TLC. After completion of the reaction, the mixture was extracted with CH₂Cl₂ (3×25 mL), and the combined organic phase was washed successively with saturated sodium chloride, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by through column chromatography (petroleum ether/ethyl acetate) to obtain the desired compound 6a-6k.

N-(pyridin-3-yl)-2-thioxothiazolidine-3-carboxamide (6g). Yellow solid powder (0.98 g, yield 64.72%), m.p. 111–113°C. ¹H-NMR δ_H (400 MHz, CDCl₃): 9.60 (s, 1H), 7.87 (m, 1H), 7.31–7.18 (m, 2H), 4.69 (t, J = 5.3 Hz, 2H), 3.40 (t, J = 9.0 Hz, 2H). ¹³C-NMR δ_C (100 MHz, DCl₃): 201.83, 150.17, 149.67, 129.55, 126.45, 121.26, 55.83, 28.43. ESI-MS: m/z calc. for C₁₀H₁₀NO₂S₂ 261.0235 [M+Na]⁺, found 261.0229 [M+Na]⁺.
2.3 Enzyme inhibitory kinetics

The inhibitory mode of representative compound 6k was further investigated using enzyme inhibitory kinetic studies. XO solution (55 μL) was mixed respectively with compound 6k (0, 5, 10, and 20 μmol/L) and incubated at 37°C for 5 min. Then, xanthine solution (0.25, 0.5, 1.0, and 2.0 mmol/L) were added to initiate the reaction at 37°C for 30 min. The reaction was carried out in a 96-well plate and the OD value were read using a microplate reader at 295 nm. The obtained data were analyzed using Microsoft Excel 2013 and transformed into Lineweaver-Burk plots using GraphPad Prism 7.0. In addition, the value of Ki and Kis could be calculated using GraphPad Prism 7.0.
2.4 Molecular modeling

The three-dimensional structure of the protein was downloaded from RCSB Protein Data Bank (www.rcsb.org) Protein Receptor with Ligand Molecule (PDB ID: 3ETR) [29], and the structure of compound 6k was constructed in the MOE module. The protein was processed using Schrödinger’s Protein Preparation Wizard [30], including removed crystal water, added missing hydrogen atoms and repaired missing bond information and peptide fragments. The Ligprep 3.3 module was used to generate stereoisomers of test compound, and the protonation states of ligands at pH 7.0 ± 2.0 were generated with Epik 3.1. Protonation and energy optimization were performed to obtain the 3D configuration using Chem3D Pro 14.0 (PerkinElmer, America). After the grid file was generated, the compound was docked using the Standard Precision mode of Ligand docking in the Glide module, and the optimal configuration was selected for force analysis and plotted with Pymol.

2.5 Statistical analysis

Statistical analyses and image processing were performed using Microsoft Excel 2013 (Microsoft Inc., America) and GraphPad Prism 7.0 (GraphPad Software Inc., America). The experiments were repeated three times, and the data in this paper were expressed as mean ± SD. The t-test was used for the comparisons between two groups, p<0.05 was considered a statistically significant difference.

3. Results and discussion

3.1 Chemical synthesis

A series of thiazolidine-2-thione derivatives were prepared in this paper, and the synthetic routes were shown in Schemes 1, 2. Thiazolidine-2-thione (3) was prepared according to previous method [31] with a slight modification using aminothanol as the starting material. Compounds 4a-4d were synthesized from thiazolidine-2-thione and ethyl bromide/bromopropane/bromobenzene/benzyl bromide by utilizing NaOH or NaOH+CuI as catalyst. Thiazolidine-2-thione was reacted with triphosgene in anhydrous CH2Cl2, and a variety of amines were added to obtain the compounds 6a-6k in the presence of triethylamine. The structures of the obtained derivatives were confirmed (S1 Fig in S1 File) by nuclear magnetic resonance (1H NMR and 13C NMR) and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS).

3.2 In vitro XO inhibitory activity

The XO inhibitory activity of all derivatives was determined by in vitro enzyme catalysis reaction with allopurinol and febuxostat as positive control, and the results were shown in Table 1. Compared with thiazolidine-2-thione (IC50 = 72.15 μmol/L), most of the derivatives showed significant XO inhibitory activity with IC50 values between 3.56 μmol/L and 58.17 μmol/L, whereas compounds 4d demonstrated weak inhibitory activity with IC50 values exceeding of 100 μmol/L. Among them, compounds (6i–6k) containing phenyl-sulfonamide in the structure exhibited the more potent XO inhibitors, revealing IC50 values of 5.19, 9.76, and 3.56 μmol/L, respectively. In particular, compound 6k exhibited an XO inhibitory activity similar to that of febuxostat (IC50 = 3.34 μmol/L), which was approximately 2.5-fold more potent than that of allopurinol (IC50 = 7.86 μmol/L). Structure-activity relationship indicated that introduction of the amide group significantly enhanced the inhibitory activity of compound (4a vs 6a; 4b vs 6b; 4c vs 6e; 4d vs 6f), which was consistent with the results of previous studies [32]. In addition, introduction of benzene sulfonamide substituted by electron-donating
group could decrease the inhibitory activity ($6i$ vs $6j$), whereas the substituted by electron-withdrawing group could obviously enhance the inhibitory activity ($6i$ vs $6k$), which meant that the electron-withdrawing group linked at the benzene sulfonyl was more preferable for the XO inhibitory activity [33].

3.3 Enzyme inhibitory kinetics analysis of compound 6k

To research the inhibitory mode of compound $6k$, enzyme inhibitory kinetic studies were performed and the inhibitory mode were analyzed using Lineweaver-Burk plots. As shown in Fig 1, the changed of compound $6k$ concentration resulted in the changes of slope and Y-intercept, suggesting that Km and Vmax were changed with the change the concentration of compound $6k$. The curve intersected in the first quadrant, showing that Km increased and Vmax decreased with the increasing of compound $6k$ concentration. The results confirmed that the mode of XO inhibition by compound $6k$ belonged to mixed competitive inhibition, which was different from allopurinol with a competitive inhibition [34]. At the same time, the values of $K_i$ and $K_{is}$ were calculated based on the Lineweaver-Burk plot, which $K_i$ was competitive inhibition constant for binding with free enzyme and $K_{is}$ was noncompetitive inhibition constant for binding with enzyme-substrate complex. The results showed that the $K_i$ and $K_{is}$ values of compound $6k$ were 7.08 μmol/L and 25.67 μmol/L, respectively, which suggested that compound $6k$ preferentially bound to the free XO rather than to the XO-xanthine complex.

3.4 Molecular modeling study of compound 6k

To further research the potential binding mode between enzyme and drug molecule, molecular docking study of thiazolidine-2-thione and compound $6k$ were performed in this paper.
Scheme 2. Synthesis of thiazolidine-2-thione derivatives 6a-6k.

i. TEA, CH₂Cl₂, 0˚C; ii. TEA, THF, R.T.

Table 1. *In vitro* XO inhibitory potency of thiazolidine-2-thione derivatives.

| Compound | IC₅₀ (μmol/L) | Compound | IC₅₀ (μmol/L) |
|----------|---------------|----------|---------------|
| 3        | 72.15 ± 1.66  | 6e       | 10.20 ± 1.37  |
| 4a       | 43.53 ± 2.18  | 6f       | 12.80 ± 1.60  |
| 4b       | 58.17 ± 6.71  | 6g       | 14.52 ± 2.90  |
| 4c       | 51.60 ± 1.64  | 6h       | 9.87 ± 1.19   |
| 4d       | >100          | 6i       | 5.19 ± 0.95   |
| 6a       | 22.90 ± 2.24  | 6j       | 9.76 ± 1.29   |
| 6b       | 27.80 ± 0.63  | 6k       | 3.56 ± 0.61   |
| 6c       | 30.54 ± 2.25  | Allopurinol | 7.86 ± 0.91 |
| 6d       | 19.04 ± 1.44  | Febuxostat | 3.34 ± 0.53  |

IC₅₀ values were expressed as the mean±SD.
Since there is no complete crystal structure and the molybdenum-pterin centers of both xanthine dehydrogenase (XDH) and XO are identical in terms of binding modes and substrate catalysis [35], so XDH crystal structure (PDB: 3ETR) was used in molecular docking research. The affinity of thiazolidine-2-thione and enzyme was -6.68 kcal/mol, and the affinity of compound 6k and enzyme was -10.3 kcal/mol, which suggested that compound 6k presented a more compact binding mode in the enzyme active pocket. As shown in Fig 2A, thiazolidine-2-thione formed a hydrogen bond with the amino acid residue Thr262 of the enzyme with a distance of 1.97 Å. Fig 2B shown that compound 6k was accommodated in the active site through hydrogen bonds with primary amino acids, including Gly260, Glu263, Ile264 and Ser347, and the interaction distances were 2.48 Å, 2.33 Å, 2.72 Å and 2.11 Å, respectively. Among them, the 4-fluorophenyl-sulfonyl moiety interacted with the amino acid residue of the enzyme active pocket via 2 hydrogen bonds, in which the carbonyl group acted as a hydrogen bond acceptor interacting with the amino group of Gly260 and Ile264, respectively. At the same time, the thiazolidinethione moiety formed two hydrogen bonds with the amino acid residue of Glu263 and Ser347 in the enzyme hydrophobic cavity, which was similar to 2-(indol-5-yl) thiazole derivatives [36]. The molecular docking results explained why compound 6k could produce more potent XO inhibitory activity than thiazolidine-2-thione.

4. Conclusion

In summary, a series of novel thiazolidine-2-thione derivatives were designed and synthesized as XO inhibitors, and the inhibitory activity was evaluated in this study. The results of in vitro enzyme catalysis shown that compound 6k was the most effective XO inhibitor with an IC₅₀ value of 3.75 μmol/L. Structure-activity relationship analysis revealed that the phenyl-sulfonamide group was indispensable for compound 6k to produce XO inhibitory activity. The enzyme inhibition kinetics analyses confirmed that compound 6k exerted a mixed-type XO inhibitor. In addition, molecular docking studies shown that compound 6k could bind tightly to the active pocket of the enzyme through hydrogen bonds, and the affinity of compound 6k and enzyme was -10.3 kcal/mol. Accordingly, the results described above suggested that compound 6k could be a valuable lead compound for the treatment of hyperuricemia.
Fig 2. Molecular docking of thiazolidine-2-thione and compound 6k within the binding pocket of XDH. (a) The binding modes of thiazolidine-2-thione with XDH (PDB: 3ETR), and the hydrogen bonds of thiazolidine-2-thione with the key amino acid residues in XDH; (b) The binding modes of compound 6k with XDH, and hydrogen bonds of compound 6k with the key amino acid residues in XDH.

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Supporting information
S1 File.
(DOCX)

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References

1. Kumar R, Joshi G, Kler H, Kalra S, Kaur M, Arya R. Toward an understanding of structural insights of xanthine and aldehyde oxidases: an overview of their inhibitors and role in various diseases. Med. Res. Rev. 2016; 38: 1073–1125. https://doi.org/10.1002/med.21457 PMID: 28672082

2. Smelcerovic A, Tomovic K, Smelcerovic Z, Petronijevic Z, Koci G, et al. Xanthine oxidase inhibitors beyond allopurinol and febuxostat: an overview and selection of potential leads based on in silico calculated physico-chemical properties, predicted pharmacokinetics and toxicity. Eur. J. Med. Chem. 2017; 135: 491–516. https://doi.org/10.1016/j.ejmech.2017.04.031 PMID: 28478180

3. Richette P, Doherty M, Pascual E, Barskova V, Uhlig T, et al. 2016 updated EULAR evidence-based recommendations for the management of gout. Ann. Rheum. Dis. 2017; 76: 29–42. https://doi.org/10.1136/annrheumdis-2016-209707 PMID: 27457514

4. Grassi W, Angelis RD. Clinical features of gout. Reumatismo 2011; 63: 238–245.

5. Kuo CF, Grainge MJ, Zhang W, Doherty M. Global epidemiology of gout: prevalence, incidence and risk factors. Nat. Rev. Rheumatol. 2015; 11: 649–662. https://doi.org/10.1038/nrrheum.2015.91 PMID: 26150127

6. Biemond P, Swaak AJ, Beindorff CM, Koster JF. Superoxide-dependent and independent mechanisms of iron mobilization from ferritin by xanthine oxidase. Implications for oxygen-free-radical-induced tissue destruction during ischaemia and inflammation. Biochem. J. 1986; 239: 169–173. https://doi.org/10.1042/bj2990168 PMID: 3026367

7. Liu C, Zhou HN, Zhang RR, Wang XK, He SW, et al. Anti-hyperuricemia and nephroprotective effect of geniposide in chronic hyperuricemia mice. J. Funct. Foods. 2019; 61: 103355.

8. Wang MX, Chen JS, Zhang RR, Guo XY, Chen YY, et al. Design, synthesis and bioactive evaluation of geniposide derivatives for antihyperuricemic and nephroprotective effects. Bioorg. Chem. 2021; 116: 105321. https://doi.org/10.1016/j.bioorg.2021.105321 PMID: 34500305

9. Shirakura T, Nomura J, Matsui C, Kobayashi T, Tamura M, Masuzaki H. Febuxostat, a novel xanthine oxidoreductase inhibitor, improves hypertension and endothelial dysfunction in spontaneously hypertensive rats. Naunyn Schmiedeberg’s Arch. Pharmacol. 2016; 389: 831–838. https://doi.org/10.1007/s00210-016-1239-1 PMID: 27198514

10. Abeles AM, Pillinger MH. Gout and cardiovascular disease: crystallized confusion. Curr. Opin. Rheumatol. 2019; 31: 118–124. https://doi.org/10.1097/BOR.0000000000000585 PMID: 30601229

11. Pacher P. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. Pharmacol. Rev. 2006; 58: 87–114. https://doi.org/10.1124/pr.58.1.6 PMID: 16507884

12. Okamoto K, Matsumoto K, Ashizawa N, Nishino T, FYX-051: a novel and potent hybrid-type inhibitor of xanthine oxidoreductase. J. Pharmacol. Exp. Ther. 2010; 336: 95–103. https://doi.org/10.1124/jpet.110.174540 PMID: 20952484

13. Chen IH, Kuo MC, Hwang SJ, Chang JM, Chen HC. Allopurinol induced severe hypersensitivity with acute renal failure. Kaohsiung J. Med. Sci. 2005; 21: 228–232. https://doi.org/10.1016/S1607-551X(09)70192-5 PMID: 15960089

14. Mari E, Ricci F, Imberti D, Gallerani M. Agranulocytosis: An adverse effect of allopurinol treatment. Ital. J. Med. 2011; 5: 120–123.

15. Bardin T, Pascart T, Filpo RM, Ea HK, Roujeau JC, Clerson P. Risk of cutaneous adverse events with febuxostat treatment in patients with skin reaction to allopurinol. A retrospective, hospital-based study of 101 patients with consecutive allopurinol and febuxostat treatment. Joint Bone Spine 2016; 83: 314–317. https://doi.org/10.1016/j.jbspin.2015.07.011 PMID: 26709250

16. Zhang TJ, Wu QX, Li SY, Wang L, Sun Q, et al. Synthesis and evaluation of 1-phenyl-1H-1,2,3-triazole-4-carboxylic acid derivatives as xanthine oxidase inhibitors. Bioorg. Med. Chem. Lett. 2017; 27: 3812–3816. https://doi.org/10.1016/j.bmcl.2017.06.059 PMID: 28693909

17. Tang HJ, Zhang XW, Yang L, Li W, Li JH, Wang JX, et al. Synthesis and evaluation of xanthine oxidase inhibitory and antioxidant activities of 2-arylenzo[b]furan derivatives based on salvianolic acid C. Eur. J. Med. Chem. 2016; 124: 637–648. https://doi.org/10.1016/j.ejmech.2016.08.019 PMID: 27614410
18. Kaur M, Kaur A, Mankotia S, Singh H, Singh A, Singh JV, et al. Synthesis, screening and docking of fused pyrano[3,2-d]pyrimidine derivatives as xanthine oxidase inhibitor. *Eur. J. Med. Chem.* 2017; 131: 14–28. https://doi.org/10.1016/j.ejmech.2017.03.002 PMID: 28286211

19. Dang Q, Liu Y, Cashion DK. Discovery of a series of phosphonic acid-containing thiazoles and orally bioavailable diamide produgs that lower glucose in diabetic animals through inhibition of fructose-1,6-bisphosphatase. *J. Med. Chem.* 2011; 54: 153–165. https://doi.org/10.1021/jm101035x PMID: 21126019

20. Wang D, Zhang Z, Lu X. Hybrid compounds as new Bcr/Abl inhibitors. *Bioorg. Med. Chem. Lett.* 2011; 21: 1965–1968. https://doi.org/10.1016/j.bmcl.2011.02.029 PMID: 21376587

21. Lee JH, Park SH, Lee H. S-acyl and N-acyl derivatives of benzothiazole-2-thiol: An example of acyl soft enolization of N-acyl oxazolidinones, oxazolidinethiones, and thiazolidinethiones. *Biometals.* 2017; 30: 841–857. https://doi.org/10.1007/s10534-017-0046-6 PMID: 28840394

22. Fei X, Gu Y, Ban Y. Thiazole orange derivatives: synthesis, fluorescence properties, and labeling cancer cells. *Bioorg. Med. Chem.* 2009; 17: 585–591. https://doi.org/10.1016/j.bmc.2008.11.083 PMID: 19103495

23. Täuscher E, Weiß D, Beckert R. Classical heterocycles with surprising properties: the 4-hydroxy-1,3-thiazoles. *Tetrahedron Lett.* 2011; 52: 2292–2294.

24. Chandrapa S, Vinaya K, Rangappa KS. Synthesis and in vitro antiproliferative activity against human cancer cell lines of novel 5-(4-methyl-benzylidene)-thiazolidine-2,4-dines. *Invest. New Drugs* 2008; 26: 437–444. https://doi.org/10.1007/s10637-008-9130-7 PMID: 18473120

25. Almeda AM, Oliveira BA. Lipophilic gold(I) complexes with 1,3,4-oxadiazol-2-thione or 1,3-thiazoline-2-thione moieties: synthesis and their cytotoxic and antimicrobial activities. *Biometals.* 2011; 47: 1791–1793.

26. Fei X, Gu Y, Ban Y. Thiazole orange derivatives: synthesis, fluorescence properties, and labeling cancer cells. *Bioorg. Med. Chem.* 2009; 17: 585–591. https://doi.org/10.1016/j.bmc.2008.11.083 PMID: 19103495

27. Lee JH, Park SH, Lee H. S-acyl and N-acyl derivatives of benzothiazole-2-thiol: An example of acyl group rearrangement. *Bull. Kor. Chem. Soc.* 2007; 28: 1211–1214.

28. Gao J, Liu XG, Zhang B, Mao Q, Zhang Z, Zou Q, et al. Design, synthesis and biological evaluation of 1-alkyl-5/6-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)-1H-indole-3-carbonitriles as novel xanthine oxidase inhibitors. *Eur. J. Med. Chem.* 2020; 190: 112077. https://doi.org/10.1016/j.ejmech.2020.112077 PMID: 32014678

29. Okamoto K, Eger BT, Nishino T, Kondo S, Pai EF, Nishino T. An extremely potent inhibitor of xanthine oxidoreductase: crystal structure of the enzyme inhibitor complex and mechanism of inhibition. *J. Biol. Chem.* 2003; 278: 1848–1855. https://doi.org/10.1074/jbc.M208307200 PMID: 12421831

30. Schrodinger Glide, LLC, New York, NY, 2016.

31. Liu YF, Liu JM, Liu XM. Reaction of 2-thiazolidinethione with halohydrocarbon: synthesis of novel N-alkylated 2-thiazolidine and S-alkylated thiazoline derivatives. *Heterocycl. Commun.* 2010; 16: 275–278.

32. Jang JW, Song JU, Kim TH, Park H, Park WS, et al. Structure-based design and biological evaluation of novel 2-(indol-2-yl) thiazole derivatives as xanthine oxidase inhibitors. *Bioorg. Med. Chem. Lett.* 2016; 26: 950–954. https://doi.org/10.1016/j.bmcl.2015.12.055 PMID: 26774578

33. Lin HC, Tsai SH, Chen CS, Chang YC, Lai ZY, Lin CM. Structure-activity relationship of coumarin derivatives on xanthine oxidase inhibiting and free radical-scavenging activities. *Biochem. Pharmacol.* 2008; 75: 1416–1425. https://doi.org/10.1016/j.bcp.2007.11.023 PMID: 18201686

34. Elion GB. Enzymatic and metabolic studies with allopurinol. *Ann. Rheum. Dis.* 1966; 25: 608–614. https://doi.org/10.1136/ard.25.6.608 PMID: 5958693

35. Enroth C, Eger BT, Okamoto K, Nishino T, Nishino T, Pai EF. Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: structure-based mechanism of conversion. *Proc. Natl. Acad. Sci.* 2006; 97: 10723–10728. https://doi.org/10.1073/pnas.97.20.10723 PMID: 11005854

36. Song JU, Choi SP, Kim TH, Jung CK, Lee JY, Jung SH, et al. Design and synthesis of novel 2-(indol-5-yl)thiazole derivatives as xanthine oxidase inhibitors. *Bioorg. Med. Chem. Lett.* 2015; 25: 1254–1258. https://doi.org/10.1016/j.bmcl.2015.01.055 PMID: 25704891