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Investigation of the interaction between benzaldehyde thiosemicarbazone compounds and xanthine oxidase

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ABSTRACT

A series of substituted benzaldehyde thiosemicarbazide compounds (**1**–**7**) were synthesized as xanthine oxidase (XO) inhibitors, and the interactions between substituted benzaldehyde thiosemicarbazide compounds (**1**–**7**) and XO were studied by ultraviolet spectroscopy, fluorescence spectroscopy, and molecular docking. It was found that the hydrogen bond and hydrophobicity were the main interactions between substituted benzaldehyde thiosemicarbazide compounds and XO, and introducing –OH at the para position of the benzene ring and a Ph- or Me-group at the amino terminal of compound **4** increased the modifier's inhibitory activity. The results suggest that the newly introduced benzene ring interacted with the hydrophobic cavity of XO by means of the π-π stacking force between the newly introduced benzene ring and the aromatic amino acid residues, such as the Phe residue, which greatly increased the modifier's inhibitory activity. We conclude that introducing the Ph-group at the amino terminal of compound **4** and the –OH group at the para position of the benzene ring was a good route to obtain novel XO inhibitors. Fluorescence spectroscopy assisted by 8-anilino-1-naphthalenesulfonic acid fluorescence probing and molecular docking were helpful for achieving a preliminary and relatively clear understanding of the interactions between target compounds and XO, which deserve further study.

1. Introduction

XO (xanthine oxidase), which is widely found in milk, human and animal cells, contains 1330 amino acids, two molecules of flavin adenine dinucleotide (FAD), two molybdenum atoms, and eight iron atoms and is a key enzyme catalyzing the conversion of xanthine or hypoxanthine to uric acid [1–4]. The molybdenum pterin co-factor is the active site in XO [5]. The iron atom in the form of a [2Fe-2S] ferredoxin iron-sulfur cluster composes part of the electron-transfer system [6]. Generally speaking, overexpression of XO activity and excessive accumulation of uric acid in the body will lead to hyperuricemia and finally evolve into gout [7]. Currently, hyperuricemia and ventilation are becoming a significant threat to human health due to negative lifestyle choices and increasing ignorance of dietary health [8]. Obviously, inhibiting XO activity and hindering the excessive accumulation of uric acid is an effective clinical means of treating gout. In fact, a large number of naturally occurring and synthesized aromatic molecules, including aromatic-cycle-containing alcohols [9], ketones [10], aldehydes [11], acids [12], esters [13], and acid amides [14], have been reported to be XO inhibitors. Unfortunately, many of them are not ideal enough, and cannot be used for clinical medicines due to their weak activity and undesirable side effects [15]. Although allopurinol is a chemically synthesized class of XO inhibitor for the clinical treatment of gout, it also has obvious side effects, e.g., fever, allergic skin rashes, diarrhea, abdominal pain, and other symptoms that can even harm the liver, which limits its application. Therefore, there is an increasing demand for efficient and feasible XO inhibitors.

Aromatic thiosemicarbazone analogues have been receiving considerable attention in the area of medicinal chemistry because of their promising biological implications and remarkable pharmacological properties [16]. Various thiosemicarbazone analogues have been used in the anti-convulsant, anti-parasitic, antioxidant, antimicrobial [17], anti-HIV-1 [18], and anti-cancer [19] treatments, in the adjuvant treatment of diabetes, and as potential tyrosinase inhibitors [20]. Overall, most reports of XO inhibitors are focused on the discovery of new bioactive compounds and investigation of their activities, whereas research on the mechanisms and interaction between XO and inhibitors are few. As we know, full and clear

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understanding of the mechanisms and interactions between XO and its inhibitors is useful for the discovery of novel potential XO inhibitors, which is of important scientific significance.

Fluorescence spectroscopy is an important and useful means of investigating the interaction between molecules and proteins [21]. In this paper, a series of benzaldehyde thiosemicarbazide compounds (**1**–**7**) were synthesized, and ultraviolet (UV) spectroscopy, fluorescence quenching, three-dimensional fluorescence, synchronous fluorescence, 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescence probe experiments, and molecular docking were used to explore the mechanisms and interactions between XO and its target compounds. Our study helps to clarify the relationship and interaction between target compounds and XO and enhances the understanding of how changing the chemical structure of target compounds affects inhibitory activity, which may provide a significant direction for obtaining novel and highly potent XO inhibitors.

2. Material and methods

2.1. Chemicals and reagents

Xanthine oxidase (EC 1.2.3.2) and its substrate xanthine were purchased from Sigma (St. Louis, MO, USA). ESI–MS data were obtained by using a Bruker ESQUIRE-LC (Germany), NMR data were acquired on a Bruker (Germany) of 400 MHz NMR spectrometer (AV400). Raw materials for synthesis of target compounds were purchased from the Sinopharm Group (Beijing, China). All other reagents were local and of analytical grade. The water used was ultra-pure water.

2.2. Synthesis of compounds

The method for synthesis of target compounds was described as reference [22]. The synthesis route of target compounds was shown in Fig. 1.

ESI–MS data were obtained by using a Bruker ESQUIRE-LC (Germany), NMR data were acquired on a Bruker (Germany) of 400 MHz NMR spectrometer (AV400).

P-nitrobenzaldehyde thiosemicarbazone (**1**): Yellow crystals, 1H NMR (400 MHz, DMSO-d6) δ 11.68 (s, 1H, NH), 8.37 (s, 2H, Ph-H), 8.31 (s, 2H, NH2), 8.19 (s, 1H, CH), 8.09 (s, 2H, Ph-H). MS (ESI): *m/z* 223.1 $[M-H]^-$

P-cyanobenzaldehyde thiosemicarbazone (**2**): White crystals, 1H NMR (400 MHz, DMSO-d6) δ 11.62 (s, 1H, NH), 8.33 (s, 1H, NH), 8.19 (s, 1H, NH), 8.04 (m, 1H, CH), 8.00 (d, 2H, Ph-H), 7.83 (d, 2H, Ph-H). MS (ESI): *m/z* 203.1 $[M-H]^-$

Enzaldehyde thiosemicarbazone (**3**): White crystals, 1H NMR (400 MHz, DMSO-d6) δ 11.40 (s, 1H, NH), 8.17 (s, 1H, NH), 8.02 (s, 1H, CH), 7.96 (d, 1H, NH), 7.77 (dd, 1H, Ph-H), 7.37 (dd, 3H, Ph-H). MS (ESI): *m/z* 178.0 $[M-H]^-$

P-hydroxybenzaldehyde thiosemicarbazone (**4**): White crystals, 1H NMR (400 MHz, DMSO-d6) δ 11.22 (s, 1H, NH), 9.84 (s, 1H, OH), 8.04 (s, 1H, NH), 7.92 (s, 1H, CH), 7.80 (s, 1H, NH), 7.58 (d, 2H, Ph-H), 6.75 (d, 2H, Ph-H). MS (ESI): *m/z* 194.0 $[M-H]^-$

M-hydroxybenzaldehyde thiosemicarbazone (**5**): White crystals, 1H NMR (400 MHz, DMSO-d6) δ 11.34 (s, 1H, NH), 9.50 (s, 1H, OH), 8.14 (s, 1H, NH), 7.94 (s, 1H, CH), 7.86 (s, 1H, NH), 7.22–7.06 (m, 2H, Ph-H), 6.78 (dd, 2H, Ph-H). MS (ESI): *m/z* 194.1 $[M-H]^-$

P-hydroxybenzaldehyde methylthiosemicarbazide (**6**): White crystals, 1H NMR (400 MHz, DMSO-d6) δ 11.25 (s, 1H, NH), 9.83 (s, 1H, OH), 8.33 (d, 1H, NH), 7.92 (s, 1H, CH), 7.59 (d, 2H, Ph-H), 6.76 (d, 2H, Ph-H), 2.97 (d, 3H, CH3). MS (ESI): *m/z* 208.1 $[M-H]^-$

P-hydroxybenzaldehyde benzil thiosemicarbazide (**7**): Gray-green crystal, 1H NMR (400 MHz, DMSO-d6) δ 11.65 (s, 1H,NH), 9.96 (s, 1H, NH), 9.91 (s, 1H, OH), 8.03 (s, 1H, CH), 7.70 (d, 2H, Ph-H), 7.54 (d, 2H, Ph-H), 7.33 (t, 2H, Ph-H), 7.17 (d, 1H, Ph-H), 6.77 (d, 2H, Ph-H). MS (ESI): *m/z* 270.0 $[M-H]^-$

2.3. XO activity assay

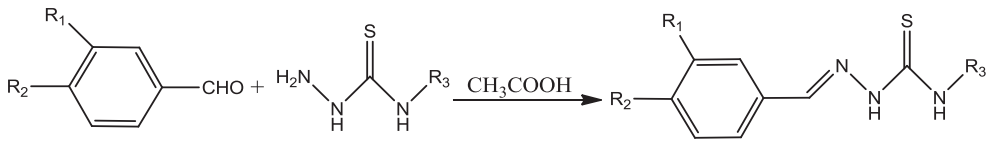
The experiment was performed by referring the previous article with minor modifications [23]. Xanthine was substrate for the assay. The reaction media (3 mL) for activity assay contained 0.85 mL 50 mM PBS buffer (Na_2HPO_4-NaH_2PO_4, pH = 7.5), 2 mL 0.15 mM xanthine in 50 mM PBS buffer (pH = 6.8) and 0.05 mL of different concentrations of inhibitor(dissolved by DMSO prior to use), and then 0.1 mL of the PBS buffer of xanthine oxidase (16.8 μg/mL) was added to the mixture. The solution was immediately monitored by measuring the linear increase in optical density at 295 nm of formation of the uric acid for 150s using a Shimadzu UV-2450 spectrophotometer (Japan). The value of inhibition ratio of compounds on XO can be calculated by the following equation:

$$\text{Inhibition rate } (\%) = (1 - OD_1/OD_2) \times 100\%$$

OD_1 is the slope of reaction kinetics equation obtained from reaction with inhibitor; OD_2 is the slope of reaction kinetics equation obtained from reaction with reagent blank. Allopurinol was used as reference standard inhibitors for comparison, respectively. The extent of inhibition by the addition of the sample was expressed as the percentage necessary for 50% inhibition (IC_{50}), calculated by SPSS19.

2.4. Measurement of inhibition mechanism and inhibition type

Inhibition mechanism was assayed by maintaining substrate concentration (0.15 mM) and increasing the concentration of XO (4.05, 8.10, 16.20 32.40 μg/mL), the value of enzyme activity was measured by adding different concentrations of target compounds



1: R_2=NO_3, R_1=R_3=H 2: R_2=C≡N, R_1=R_3=H
3: R_1=R_2=R_3=H 4: R_2=OH, R_1=R_3=H
5: R_1=OH, R_2=R_3=H 6: R_1= H, R_2=OH, R_3=Me
7: R_1= H, R_2=OH, R_3=Ph

Fig. 1. Synthesis route of benzaldehyde thiosemicarbazones.