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Antihyperuricemic effects of thiadiazolopyrimidin-5-one analogues in oxonate treated rats

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ABSTRACT

Hyperuricemia is a risk factor for not only gout, but also to a variety of disorders that affect the vital organ systems of the human body. The xanthine oxidase (XO) is the key enzyme in the production of uric acid and its inhibition can inhibit hyperuricemia. Although, XO inhibitor allopurinol is widely prescribed antigout agent but its use is not without any side effects. Previously, we described the synthesis of four novel thiadiazolopyrimidin-5-one analogues as effective XO inhibitors and molecular docking studies also confirmed this. When these analogues were tested in potassium oxonate treated rats, their serum uric acid and creatinine levels were dropped significantly from 4.85 ± 0.03 mg/dl to 1.21 ± 0.01 mg/dl and 0.92 ± 0.02 mg/dl to 0.40 ± 0.02 mg/dl respectively. Among the pyrimidine analogues tested, **6a** was most potent. Histological examinations of both liver and kidney tissues exhibited severe necrosis in oxonate treated rats and pyrimidine analogues could significantly attenuate this with a correlative inhibitory profile of hepatic XO from the same rats. Our results demonstrate antihyperuricemic effect of novel thiadiazolopyrimidin-5-one analogues in oxonate treated rats, which can be further explored not only as antigout therapeutics but also in other systems where hyperuricemia is the driving cause of the disease.

1. Introduction

Hyperuricemia (>7 mg/dl of uric acid in human serum) is a disorder resulting from the overproduction or under excretion of uric acid and is an established risk factor for 'gout' (Choi et al., 2005; Duskin-Bitan et al., 2014). Elevated uric acid levels are also associated with a range of disorders that include renal dysfunction, cardiovascular diseases, hypertension, diabetes (Dalbeth and So, 2010; Choi and Ford, 2007; Johnson et al., 2005; Short and Tuttle, 2005), cancer, obesity, and hyperlipidemia (Lin et al., 2000). High dietary intake of nucleic acids also results in hyperuricemia (Choi et al., 2004). The increase in uric acid concentration in the body eventually leads to the deposition of monosodium urate crystals in the joints and kidneys leading to inflammation as well as gouty arthritis and uric acid nephrolithiosis (Kramer and Curhan, 2002; Tomita et al., 2000; Huang et al., 2011). Compounds that display the ability to enhance the urinary excretion of uric acid or those that inhibit the uric acid biosynthesis have been employed for the treatment of gout (Ishibuchi et al., 2001).

Uric acid is formed by the oxidation of hypoxanthine to xanthine and then to uric acid by the enzyme xanthine oxidase (XO) (Okamoto, 2008). This reaction also produces reactive oxygen species (ROS) that initiate tissues damage in a range of pathophysiological states (Bonomini et al., 2008). Therefore, XO inhibitors such as allopurinol are in use in treating both hyperuricemia and other related disorders where ROS are implicated (Riegersperger et al., 2011). However, use of allopurinol is associated with undesired effects such as gastrointestinal upset, skin rashes, hypersensitivity reactions, liver dysfunction, exfoliative dermatitis, vasculitis, eosinophilia, acute interstitial nephritis (Emmerson., 1996; Wang et al., 2004), Stevens-Johnson syndrome, renal toxicity and fatal liver necrosis (Wallach, 1998). Recently discovered drug febuxostat, an alternative to allopurinol also showed higher incidence of hepatotoxicity in clinical trials (Hair et al., 2008).

We had previously described the synthesis of twenty pyrimidine analogues, among which four of them (**6a, 6b, 6d** and **6f**) showed inhibition against XO using three XO sources (Sathisha et al., 2011). Docking studies showed important interactions of these molecules in the active site of XO (Sathisha et al., 2011). In the present study, we report antihyperuricemic effects of these synthetic analogues in potassium oxonate treated rats.

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2. Materials and methods

2.1. Chemicals

Xanthine, allopurinol and potassium oxonate (oxonic acid potassium salt) were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other chemicals and reagents, which were used in this study were of analytical grade.

2.2. Preparation of test samples

The detailed synthesis of the test compounds [7-methyl-2-(phenoxymethyl)-5H[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one derivatives of **6a** (2-((4-Methoxyphenoxy)methyl)-7-methyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one), **6b** (2-((4-Chlorophenoxy)methyl)-7-methyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one), **6d** (7-Methyl-2-((o-tolyloxy)methyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one) and **6f** (2-((4-chloro-3-methylphenoxy)methyl)-7-methyl-5H-[1,3,4] thiadiazolo[3,2-a]pyrimidin-5-one) compounds] is reported in our previous communication (Sathisha et al., 2011). These compounds were dissolved in dimethyl sulfoxide (DMSO) at the concentration of 1 mg/ml.

2.3. Animals

Male wistar rats (8 – 10 weeks old) were obtained from the central animal facility, Department of Studies in Zoology, University of Mysore, after obtaining the ethical clearance from the Institutional Animal Ethical Committee (IAEC) of University of Mysore (UOM/IAEC/17/2011). The rats were housed on a constant 12 h light/12 h dark cycle in a temperature and humidity controlled room and were allowed free access to food and water.

2.4. Induction of hyperuricemia in rats

Rats were randomly divided into twelve groups: Group 1 received DMSO solution (vehicle control). Group 2 was administered with potassium oxonate (250 mg/kg)-an uricase inhibitor, injected intraperitoneally to overnight fasted rats to increase serum urate levels and served as hyperuricemic rats. Group 3 was administered with standard drug allopurinol (10 mg/kg) alone. Hyperuricemic rats receiving allopurinol were put under group 4. The groups 5 – 12 were all hyperuricemic rats receiving four test compounds at two doses (**6a, 6b, 6d** and **6f** at 50 and 100 mg/kg respectively). The test compounds were dissolved in DMSO and were administered intraperitoneally to the rats, 1 h after the potassium oxonate injection (Nepali et al., 2011a; B-Rao et al., 2012).

Blood was collected from the rats by cardiac puncture 2 h after the administration of the test compounds and were killed after the blood collection. Kidney and liver tissues were collected and used for histological/enzyme analyses. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuged at 1000 g for 20 min to obtain the serum. The serum was stored at −20 °C until assayed.

2.5. Measurement of serum uric acid and creatinine levels

Serum uric acid and creatinine levels were determined by using assay kits from Randox (Bangalore, India) in an autoanalyzer (Beckman-Coulter, AU480 ISE) as per manufacturer's instructions. The results were expressed in mg/dl.

2.6. Assay for XO activity in rat liver

XO activity was assayed by monitoring uric acid formation using a spectrophotometric method described previously (Sathisha et al., 2011; Kadam and Iyer, 2007). Briefly, 2 g of the liver from respective groups of rats was homogenized (Remi, India) with 5 volumes of 10 mM Tris–HCl buffer, pH 8.0 containing 1 mM EDTA. The homogenate was then heated to 55 °C for 5 min and then cooled rapidly on an ice bath with constant stirring. It was then subjected to centrifugation at 16,000g for 15 min. Ammonium sulphate was added to the resulting supernatant to a final concentration of 30% saturation and the mixture was centrifuged at 16,000g for 15 min. XO in the supernatant was precipitated by further addition of ammonium sulphate to a final concentration of 60% saturation. The resulting precipitate was collected by centrifugation at 16,000g for 15 min and the pellet was resuspended in 50 mM potassium phosphate buffer, pH 7.5 containing 300 mM EDTA and stored at −80 °C until use.

The enzyme assay mixture consisted of 50 mM potassium phosphate buffer (pH 7.5) containing 300 mM EDTA and the enzyme source from the respective treatment groups in a total volume of 2 ml. The reaction was initiated by the addition of xanthine (50 μM) as the substrate to the above assay mixture. The change in absorbance at 292 nm was recorded in a spectrophotomenter (Biomate 3 s, Thermo scientific, USA). The absorption rate at 292 nm indicates the formation of uric acid. The assays were carried out in duplicates and were repeated three to four times. The protein concentration of the tissue homogenates were determined by the Lowry's method, using bovine serum albumin as standard (Lowry et al., 1951) to normalize the specific activity of XO. XO specific activities were expressed as nmoles of uric acid formed/min/mg protein. The percentage inhibition of XO activity was calculated after addition of tested compounds.

2.7. Histological analyses of kidney and liver

Tissues collected from the above experimental rats were fixed in Bouine's solution (picric acid: formaldehyde: glacial acetic acid 30:10:2) for 24 h and then subjected to dehydration with increasing concentrations of ethanol and embedded in paraffin wax. Sections of 5 μm were taken using microtome (R. Jung AG, Germany) and stained with classical hematoxylin and eosin staining protocol.

2.8. Statistical analysis

The data were expressed as mean ± S.E.M. Statistical comparisons were performed by one way ANOVA.

3. Results

XO is a one of the key enzyme in purine catabolism. The elevated activity of this enzyme leads to hyperuricemia. Therefore, traditionally inhibiting this enzyme by drugs such as allopurinol is a way to control hyperuricemia. However, undesired effects associated with the use of allopurinol has forced scientific community to formulate new chemical entities. Previously, we had reported the synthesis of twenty pyrimidine analogues (7-methyl-2-(phenoxymethyl)-5H [1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one derivatives) of which 4 showed significant XO inhibitory activity (**6a, 6b, 6d** and **6f**) (Sathisha et al., 2011). The structures of these analogues are shown in Fig. 1.

3.1. Effect of test compounds on serum uric acid and creatinine levels in oxonate treated rats

Administration of the uricase inhibitor-potassium oxonate resulted in significantly increased serum uric acid and creatinine levels when compared to the control group (Table 1). Basal serum