Bioorganic Chemistry 79 (2018) 201–211

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis, molecular docking and xanthine oxidase inhibitory activity of 5-aryl-1*H*-tetrazoles

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ARTICLE INFO

Keywords:
5-Aryl-1*H*-tetrazoles
Xanthine oxidase Inhibition
Hyperuricemia
Gout
Uric acid
Structure-activity relationship

ABSTRACT

5-Aryl-1*H*-tetrazoles (**1–24**) were synthesized and screened for their xanthine oxidase (XO) inhibitory activity using allopurinol as standard inhibitor (IC_{50} = 2.0 ± 0.01 μM). Six compounds **3**, **4**, **5**, **9**, **21**, and **24** exhibited significant to weak activities with IC_{50} values in the range of 7.4–174.2 μM. Active compounds were further subjected to kinetic and molecular docking studies to deduce their modes of inhibition, and to study their interactions with the protein (XO) at atomic level, respectively. Interestingly, all these compounds showed a competitive mode of inhibition. Docking studies identified several important interactions between the ligand and the receptor protein (XO). Some of these interactions were similar to that exhibited by clinical inhibitors of XO (allopurinol, and febuxostat). This study identifies 5-aryl-1*H*-tetrazoles as a new class of xanthine oxidase inhibitors, which deserves to be further, investigated for the treatment of hyperuricemia and gout.

1. Introduction

Tetrazoles are heterocyclic compounds containing four nitrogen atoms in a five membered ring. Tetrazole ring is a stable substitute of carboxylic group, since both have very close acid dissociation constant (K_a) values. Improved biological activities were found when *C*-terminal amino acid residue was replaced with a tetrazole group in peptide inhibitors (KMI-358 and KMI-370) of Alzheimer's *β*-secretase (BACE1) [1–3]. Tetrazoles have applications in organometallic chemistry as peptide chelating agents [4]. They are also used as gas generating agents for air bags in automobiles [5]. Since tetrazoles may be converted to corresponding amides, therefore, other classes of compounds, such as aziridines, diaziridines, isourea, oxadiazole, *etc,* can be prepared from them. For the same reasons, tetrazoles are considered as an important class of precursors for the synthesis of nitrogen and sulfur containing heterocyclic compounds [6].

Tetrazole class of compounds has received major attention of medicinal chemists due to its wide applications in the treatment of central nervous system (CNS) disorders [7], HIV/AIDS [8], sexual dysfunctions [9–11], asthma [12], obesity, and diabetes [13–15]. Some of the derivatives of tetrazoles have antihypertensive, antiallergic, and antibiotic properties [16,17]. Anti-inflammatory activities of *N*-substituted tetrazoles were studied by protein (albumin) denaturation mechanisms [18]. A few derivatives of tetrazoles, as sodium-glucose co-transporter 2 (SGLT2) inhibitors, possess hypoglycemic activities [19]. During the current study 5-aryl-1*H*-tetrazoles were synthesized as potential inhibitors of xanthine oxidase.

Xanthine oxidoreductase (XOR) is a homodimer with four redox centres and some 1330 amino acid residues. Significant homology has been observed in XO from various sources. For instance, bovine milk xanthine oxidase (1332 residues) is 90% homologous to human liver enzyme (1333 residues). The key difference between xanthine oxidase (XO) and xanthine dehydrogenase (XDH) forms is in the FAD binding domain, while the xanthine binding site is identical in the two forms [20–23].

XO is a key enzyme which plays a major role in the oxidation of hypoxanthine to xanthine, and then xanthine to uric acid, which is excreted out from the body [24–26]. Overproduction of uric acid in the body leads to hyperuricemia, which is also linked with gout. Gout is a metabolic disease in which excessive levels of uric acid cause deposition

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https://doi.org/10.1016/j.bioorg.2018.04.021
Received 7 October 2017; Received in revised form 20 April 2018; Accepted 24 April 2018
Available online 01 May 2018
0045-2068/

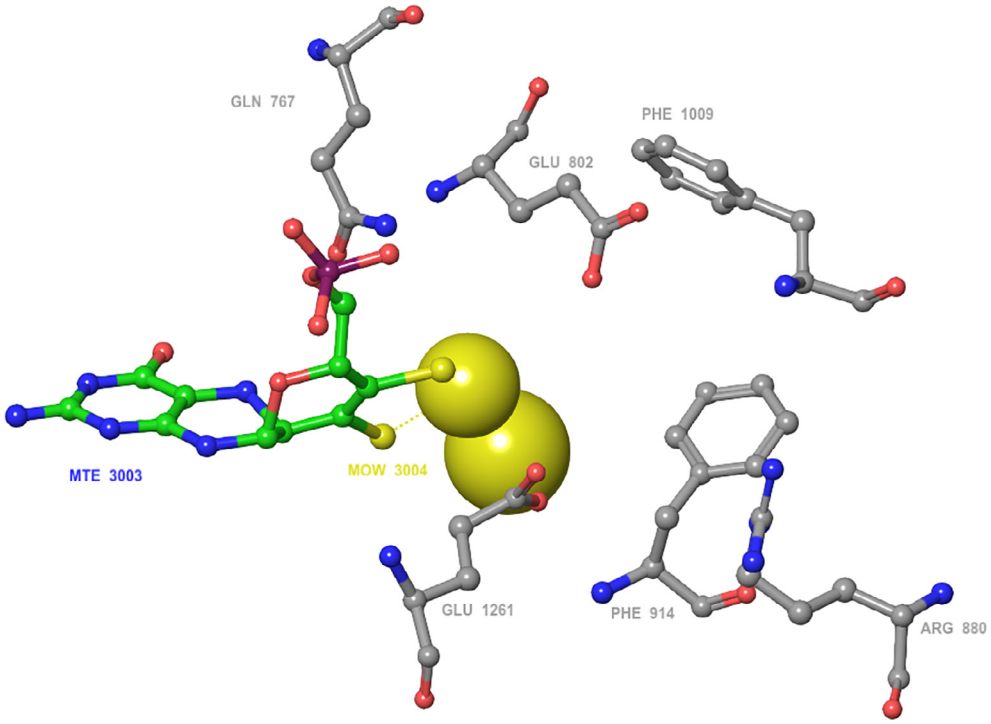

Fig. 1. Catalytically important amino acid residues in the active site of xanthine dehydrogenase (PDB ID: 3BDJ). Amino acid residues are shown in grey color, and labelled with names and positions. Molybdopterin (Mo) cofactor is shown in green color (ball and stick model) with Mo atom in yellow (CPK model).

of urate crystals in joints, resulting in inflammation [27–29]. Abnormal activity of xanthine oxidase enzyme is also associated with other diseases, such as respiratory syndrome, viral infection, hepatitis, inflammation, ischemia-reperfusion, carcinogenesis, and ageing. Uric production in the body can be lowered by xanthine oxidase inhibitors. Allopurinol, febuxostat, and recently developed topiroxostat are the clinical inhibitors of XO, used for the treatment of hyperurecemia and gout [30–33].

Computational studies showed the role of specific amino acid residues in xanthine oxidase catalyzed reaction (Fig. 1). These include Glu1261 that acts as a nucleophile, two Phe residues (914 and 1009) that surround the substrate, Gln767 which interacts with the Molybdoprotein, and Arg880 which interacts with the distal end of the substrate through hydrogen bonding. Oxypurinol (the active metabolite of allopurinol) interacts covalently with Mo, and non-covalently with Glu802, Glu1261, and Arg880, thereby inhibiting the enzyme activity [34,35].

Okamato et al. [36] in 2013 reported the crystal structure of bovine milk XDH bound with TEI-6720 (febuxostat). They highlighted some other important amino acid residues that occupy a narrow access channel that leads towards the Mo center in the active site of XO. These include Phe (649, 914, 1009, 1013), Leu (648, 649, 1014), and Val1011. Febuxostat, which is a clinical inhibitor of XO, fill this narrow channel and thereby block the entry of substrate into the active site (Fig. 2) [37].

Current inhibitors of XO have several adverse effects, and there is a need of new XO inhibitors with better efficacy, and lower side effects [38–40]. Recently, we have reported pyrimidine- [41] and quinazoline- [42] based compounds as xanthine oxidase inhibitors *in vitro*. The observed XO inhibitory activity of nitrogenous derivatives motivated us to synthesize a class of nitrogen loaded heterocycle tetrazoles for xanthine oxidase inhibitory evaluation. Some encouraging results were observed (Table 1), which are discussed in the forthcoming paragraphs. Compounds **1**, and **5** are new compounds, while the remaining compounds were identified as previously reported compounds [43–61].

2. Results and discussion

2.1. Chemistry

Twenty-four derivatives of 5-aryl-1*H*-tetrazole (**1–24**) were synthesized by mixing sodium azide, ammonium chloride, and differently substituted nitrile derivatives in dimethyl formamide (DMF) under refluxing conditions (Scheme1). Precipitation occurred upon addition of aqueous NaOH, followed by acidification with HCl. The precipitates were filtered, washed with water, and dried under vacuum. ^{1}H NMR and EI mass spectrometry was used to deduce the structures of the desired products. All synthesized compounds showed satisfactory HREI-MS data.

2.2. Enzyme inhibitory studies

All synthetic 5-aryl-1*H*-tetrazoles (**1–24**) were evaluated for their xanthine oxidase (XO) inhibitory activity. Compounds **9** (IC_{50} = 7.4 ± 0.01 μM), **24** (IC_{50} = 14.1 ± 0.04 μM), **21** (IC_{50} = 43.5 ± 2.5 μM), **5** (IC_{50} = 51.8 ± 1.4 μM), **4** (IC_{50} = 110.23 ± 1.0 μM), and **3** (IC_{50} = 174.2 ± 1.98 μM) showed significant to weak inhibition of the enzyme, when compared to the standard, allopurinol (IC_{50} = 2.0 ± 0.01 μM). All other compounds were found to be inactive (Table 1).

Parent compound **8**, with no substitution on the aryl ring, was found to be inactive. Structure-activity relationship of 5-aryl-1*H*-tetrazoles derivatives **1–24** revealed that their XO inhibitory activity depends on different substituents on the aryl part of the 5-aryl-1*H*-tetrazole skeleton.

Compound **9** (IC_{50} = 7.4 ± 0.01 μM) was a significantly active XO inhibitor in this series in comparison to the standard drug, allopurinol (IC_{50} = 2.0 ± 0.01 μM). The electron withdrawing nitro group at C-4′ (*para*) apparently made the tetrazolic proton quite acidic to be engaged in hydrogen bonding with the amino acid residues of the enzyme. Compound **11**, with a nitro group at C-3′ (*meta*), was found to be inactive, indicating that the position as well as the substitutents (nitro in this case) are important for the inhibitory activity. The placement of