Revista Brasileira de Farmacognosia 26 (2016) 50–55

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.journals.elsevier.com/revista-brasileira-de-farmacognosia

Original Article

Screening of ferulic acid related compounds as inhibitors of xanthine oxidase and cyclooxygenase-2 with anti-inflammatory activity

Shivraj Hariram Nile, Eun Young Ko, Doo Hwan Kim, Young-Soo Keum *

Department of Bioresources and Food Science, College of Life and Environmental Sciences, Konkuk University, Seoul, South Korea

ARTICLE INFO

Article history:
Received 6 May 2015
Accepted 23 August 2015
Available online 29 November 2015

Keywords:
Ferulic acid
Gallic acid
Xanthine oxidase
Cyclooxygenase
SAR
Gout

ABSTRACT

The ferulic and gallic acid related compounds from natural origin were studied against xanthine oxidase and cyclooxygenase-2 along with their anti-inflammatory activity. The compounds gallic acid, ferulic acid, caffeic acid and *p*-coumaric acid revealed promising anti-inflammatory activity (30–40% TNF-α and 60–75% IL-6 inhibitory activity at 10 μM). Bioavailability of compounds were checked by *in vitro* cytotoxicity using CCK-8 cell lines and confirmed to be nontoxic, but found toxic at higher concentration (50 μM). Gallic, ferulic, caffeic acid was demonstrated potential dual inhibition toward xanthine oxidase and cyclooxygenase-2 as calculated by IC_{50}: 68, 70.2, and 65 μg/ml (xanthine oxidase) and 68.5, 65.2, and 62.5 μg/ml (cyclooxygenase-2), respectively. The structure activity relationship and *in silico* drug relevant properties (HBD, HBA, PSA, *c* Log P, ionization potential, molecular weight, E_{HOMO} and E_{LUMO}) further confirmed that the compounds were potential candidates for future drug discovery study, which was expected for further rational drug design against xanthine oxidase and cyclooxygenase.

Introduction

Gouty arthritis is a well known disease with an abrupt attack causing extreme pain in and around the joints due to activities occurred by xanthine oxidase (Mohapatra et al., 2015). Gout is nearly always associated with chronic hyperuricemia, a long-lasting abnormally high concentration of uric acid (hyperuricemia) in the blood. The higher levels of uric acid can reach a point where uric acid crystals (monosodium urate) are put differently and this hyperuricemia results in the deposition of crystals of sodium urate in tissues, especially in kidneys and joints (Bardin, 2004; Choi and Curhan, 2005). This process generates oxygen metabolites, which damage tissue, resulting in the release of lysosomal enzymes that induce an inflammatory response. This will lead to local decrease of pH which further causes more deposition of urate crystals (Nuki and Simkin, 2006). The diagnosis of gout is based on the presence of monosodium urate crystals in the synovial fluid (Martinon and Glimcher, 2006). This deposition will exacerbate leading to recurrent episodes of acute arthritis, the classic manifestation of gout. Meanwhile, controlling the uric acid level in the blood is still the main target particularly in the management of the chronic attacks (Mandell, 2002; Dincer et al., 2002). Xanthine oxidase (XO) has a major role in the uric acid production as XO is responsible for catalyzing the oxidation of hypoxanthine to form xanthine and finally to uric acid. Thus, this enzyme coordinates the reaction and produces uric acid from its precursors (Nile et al., 2013; Li et al., 2013). Similarly, certain enzymes such as cyclooxygenase-2 (COX-2) contributes its role in the gouty inflammation, though it heightened expressions in the presence of the accumulated MSU crystals, which in turn enhances the production of inflammatory prostaglandins leading to the increased production of IL-1β. Thus, COX-2 plays a major role in arousing the inflammatory responses and thus taking part in the advancement of the acute inflammation in the gouty arthritis patients (Pouliot et al., 1998; Mohapatra et al., 2015). There are certain measures taken in order to treat the disease by using non-steroidal anti-inflammatory drugs (NSAID), colchicine or gluco corticoids. NSAID are the class of drugs which causes COX-2 inhibition whereas colchicine is an antimycotic alkaloid that apart from disturbing the microtubule polymerization, it also inhibits the inflammation by preventing the IL-1β processing which was stimulated by the MSU crystals (Martinon et al., 2006; Ricciotti and FitzGerald, 2011). Examples of such drugs are allopurinol, probenecid and sulfinpyrazone (Mandell, 2002; Aggarwal et al., 2011). Modern medicines from natural products have little to offer for alleviation of gout, oxidative and inflammatory activity. There is an urgent need to discover compounds with xanthine oxidase inhibitory activities but devoid of the undesirable effects of allopurinol. One potential source of such compounds is medicinal

* Corresponding author.
E-mail: rational@konkuk.ac.kr (Y.-S. Keum).

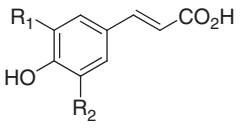
http://dx.doi.org/10.1016/j.bjp.2015.08.013
0102-695X/

materials of plant origin which is used to treat conditions similar to gouty arthritis (Nile and Park, 2014a,b). Phenolics and flavonoids are considered important components in the human diet because of their beneficial effects on human health. The phenolics and flavonoids are naturally occurring compounds that are constantly distributed in various foods, fruit juices and beverages from plant origin and display many bioactive and therapeutic properties, such as antioxidant, anticancer, antiviral, anti-inflammatory, and cardiac protective effects (Proestos et al., 2005; Nile and Park, 2014c). Most of the therapeutic properties of phenolics and flavonoids have been demonstrated to have potent antioxidant, anti-inflammatory and enzyme inhibition properties. Several phenolics and flavonoids have been described as inhibitors of the xanthine oxidase (XO) enzyme, similar to allopurinol in the treatment of gout (Bandgar et al., 2009; Nile and Park, 2013), for this reason, research into ferulic acid seems promising. Thus, the aim of this study is to investigate the potency of naturally occurring ferulic acid related compounds as xanthine oxidase and cyclooxygenase-2 inhibitors along with the properties like anti-inflammatory activity, structure activity relationship (SAR) and *in silico* drug relevant properties were studied.

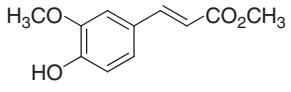
Materials and methods

Chemicals

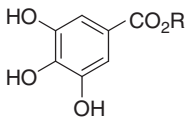
Xanthine oxidase, xanthine, allopurinol, myricetin, and isoquercetin were purchased from Sigma Chemical (Seoul, Korea). Ferulic acid (**2**), gallic acid (**6**), and their esters were supplied by Hi-Media Laboratories, Mumbai, India. Caffeic acid (**3**), sinapic acid (**4**), *p*-coumaric acid (**1**) procured from Sigma–Aldrich, Mumbai, India. Cyclooxygenase fluorescent inhibitor screening assay kit was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Bovine milk xanthine oxidase procured from (grade 1, ammonium sulfate suspension) Sigma–Aldrich, Mumbai, India.



1 R_1=R_2=H
2 R_1=OCH_3; R_2=H
3 R_1=OH; R_2=H
4 R_1=R_2=OCH_3



5



6 R=H
7 R=CH_3
8 R=$(CH_2)_2CH_3$
9 R=$(CH_2)_{11}CH_3$
10 R=$(CH_2)_{17}CH_3$

Anti-inflammatory and cytotoxicity assay

Pro-inflammatory cytokine production by lipopolysaccharide (LPS) in THP-1 cells was measured according to the method described by Bandgar et al. (2009). During assay, THP-1 cells were cultured with penicillin and streptomycin (100 U/ml) and inoculated with 10% fetal bovine serum (FBS, JRH) in RPMI 1640 culture medium (Gibco BRL, Pasley, UK). Cells were differentiated with phorbol myristate acetate (PMA, Sigma). Following cell plating, the test compounds (10 μM) in 0.5% DMSO was poured to each well and the plate were incubated for 30 min at 37 °C. Finally, LPS (*Escherichia coli* 0127:B8, Sigma Chemical Co., St. Louis, MO) was added, at a final concentration of 1 μg/ml in each well. Plates were further incubated at 37 °C for 24 h in 5% CO_2. After incubation, supernatants were harvested, and assayed for TNF-α and IL-6 by ELISA as described by the manufacturer (BD Biosciences, India). The cells were simultaneously evaluated for cytotoxicity using CCK-8 from Dojindo Laboratories. Percent inhibition of cytokine release compared to the control was calculated. In this the cytotoxicity was checked at lower, optimum and higher concentration (10, 25 and 50 μM), 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method (Bandgar and Gawande, 2010; Nile and Khobragade, 2011).

In silico *pharmacological property and SAR study*

The pharmacological properties of the compounds, such as molecular weight, *c* Log P and quantum chemical descriptors such as E_{HOMO} (Energy of highest occupied molecular orbital) and E_{LUMO} (energy of lowest unoccupied molecular orbital) of the synthesized compounds were calculated using a BioMed CaChe 6.1 (FujiSuit Ltd), a computer aided molecular design modeling tool for windows 98/20000/XP operating system. Other parameters such as HBA (hydrogen bond acceptor), HBD (hydrogen bond donor), molecular PSA (polar surface area), drug score and drug likeness of the compounds were also studied using online Osiris property explorer for drug bioavailability of chemical compounds. Since compounds are considered for oral delivery, they were also assessed for toxicity using *in silico* ADME prediction methods (Bandgar and Gawande, 2010; Nile and Khobragade, 2011).

XO inhibitory activity

Xanthine oxidase (XO) activity was assayed spectrophotometrically by measuring the uric acid formation at 290 nm using a UV-visible spectrophotometer at 25 °C. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.6), 75 μM xanthine and 0.08 units of XO. Inhibition of XO activity of individual isolated phenolics from maize (1.5 ml, 2 mM), was measured by following the decrease in the uric acid formation at 293 nM at 25 °C. The enzyme was pre incubated for 5 min, with test compound, dissolved in DMSO (1%, v/v), and the reaction was started by the addition of xanthine. Final concentration of DMSO (1%, v/v) did not interfere with the enzyme activity. The XO kinetic study was carried out using screening of ten ferulic acid related compounds (10, 25, 50 and 100 μg/ml) comparing with allopurinol (10, 25, 50 and 100 μg/ml) as positive control. All the experiments were performed in triplicates and IC_{50} values were expressed as means of three experiments (Nile et al., 2013; Nile and Park, 2014a).

COX-2 inhibitory activity

Cayman's COX fluorescent inhibitor screening assay provides a convenient fluorescent-based method for screening both ovine COX-1 and human recombinant COX-2 for isozyme-specific inhibitors. The assay utilizes the peroxidase component of COX. The reaction between prostaglandin- G2 and 10-acetyl-3, 7-dihydroxyphenoxazine produces the highly fluorescent compound resorufin. Resorufin fluorescence can be easily analyzed with an excitation wavelength of 540 nm and an emission wavelength of