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Review Article

EXPLORATION OF MICROORGANISMS AS A POTENTIAL SOURCE OF XANTHINE OXIDASE INHIBITORS: AN UPDATED REVIEW

UMA RAJESWARI BATCHU[a*], JOSHNA RANI SURAPANENI[a]

[a]Department of Pharmaceutics, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Viswavidyalayam, Tirupati, 517502, India
Email: umarajeswaribatchu@gmail.com

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ABSTRACT

Nowadays the prevalence of hyperuricemia has significantly increased in which serum uric acid levels are exceeding the normal range. Gout is the predominant clinical implication of the hyperuricemia, but many clinical investigations have confirmed that hyperuricemia is an independent risk factor for cardiovascular disease (CVD), hypertension, diabetes, and many other diseases. The xanthine oxidase (XO) converts hypoxanthine to xanthine and ultimately to uric acid, and the irreversibly accumulated uric acid causes hyperuricemia associated with gout. Hence specific and selective xanthine oxidase inhibitors (XOI) are potentially powerful tools for inactivating target XO in the pathogenic process of hyperuricemia (Gout). The objective of the current study was to overview the various XOI isolated from the microorganisms. Microorganisms have been employed for several decades for the large-scale production of a variety of bio-chemicals ranging from alcohol to antibiotics and as well as enzyme inhibitors. Currently available XOI (allopurinol and febuxostat) for the treatment of gout have been exhibiting serious side effects. Thus, there is a need to search for new molecules to treat hyperuricemia and its associated disorders. At present, microbes have been unexplored in the development of successful products for the management of XO-related diseases. Hence, the present review focused on novel XOI produced from various microbial species such as Actinobacteria, lichens, bacteria, endophytic fungi and mushrooms, which can be expected to play an important role in the ongoing transition from the empirical screening to the real rational drug design.

Keywords: Xanthine oxidase inhibitors, Hyperuricemia, Actinobacteria, Bacteria, Fungi, Lichens

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INTRODUCTION

Hyperuricemia has long been established as the major etiological factor in various disorders such as gout, urolithiasis, chronic kidney disease (CKD), tumor lysis syndrome (TLS) and various cardiovascular diseases (CVD) [1, 2]. Hyperuricemia results due to high serum urate levels, which is attributed to its overproduction or underexcretion. Gout remains the most common among all pathologies associated with hyperuricemia. In India, approximately 0.12-0.19% population being affected by gout, and its prevalence has been more in men aged above 50 y than premenopausal women as estrogen hormone helps in urate clearance [3, 4]. Gout was described by Hippocrates as "The disease of kings" owing to its association with a rich diet [5]. The xanthine oxidoreductase (XOR) is generally recognized as the key factor in hyperuricemia, recognized as the terminal enzyme of purine catabolism in humans. Mammalian XO (XO; EC 1.1.3.22) and xanthine dehydrogenase (XDH) (XDH; EC 1.1.1.204) are interconvertible forms of the same gene product known as XOR catalyzing the hydroxylation of hypoxanthine to xanthine and xanthine to uric acid [6-8]. This process is a source of reactive oxygen species (ROS) as a byproduct of uric acid, which being associated with diverse pathological events including inflammation, metabolic disorders, cellular aging, atherosclerosis, Parkinson's disease, Alzheimer's dementia, reperfusion injury of brain or heart and carcinogenesis [9-11]. The detailed structure, physiological and pathological role of XOR was reviewed previously [12]. The strategy involves the inhibition of the XO appears to be safer as it inhibits circulating levels of uric acid as well as vascular oxidative stress and associated disorders. Till date, only allopurinol, a purine analog, Febuxostat and Topiroxostat, non-purine based selective inhibitors have been clinically approved as XO inhibitors [13]. Unfortunately, allopurinol is being associated with an infrequent but severe hypersensitivity [14]. Clinically non-purine analogs (Febuxostat and Topiroxostat) provides greater hyperuricemic activity and less toxicity than allopurinol. However, hypersensitivity reactions of febuxostat also reported [15]. Nevertheless, topiroxostat exhibits high bioavailability and safety in humans, but side effects are not well explored owing to the short duration of clinical use in Japan [16]. In view of the drawbacks of existing therapies, there is a need to develop novel selective inhibitors of XO. Plants have served as an excellent source of novel medicinal compounds [17]. However, the inherent bottleneck encountered by the pharmaceutical industry in their bulk production as it requires huge quantities of biomass for their extraction which eventually threatens the existence of these plants. Moreover, enzyme inhibitors isolated from microbial sources are potent low molecular weight compounds derived from the hydrolysis of macromolecular substances when compared to inhibitors derived from plants and animals [18].

Several review articles focusing on the XOI of diverse sources such as plants, synthetic analogs have been published, serving as a starting point for exploration [19-23]. Thus the alternative resources were embattled to substitute these Phyto medicinals. It has been amply demonstrated by various studies that the metabolites produced by microorganisms, in particular, are recognized as a resource for numerous therapeutic moieties [24]. Therefore the objective of our present review is to summarize the various XOI derived from microbial origin. This is the first review exploring the updates of various XOI from microorganisms.

XOI from various microbial sources

Since several decades XOI has been screened and synthesized from various sources (fig. 1). Selected microorganisms, including bacteria, fungi, and yeasts have been globally studied for the bio-synthesis of economically valuable preparations of various enzymes and enzyme inhibitors for commercial applications [25, 26]. In view of their medicinal potential, they have been screened for the isolation of XOI.

Bacteria

Two specific XOI has been isolated from the culture filtrate of *Alcaligenes aquamarinus* No.655, and *Bacillus cereus* No. A-73 strains [27]. One among is identified as 2,8-dihydroxy adenine which was synthesized newly by microbial route (*Alcaligenes aquamarinus* No.655), but previously by chemical route [28]. The inhibitor has

shown 50% inhibition at a concentration of 3 x 10-6 M (0.5 g/ml*)* on rat liver XO and 2 x 10-6 M (0.33 g/ml) on milk XO, respectively. As well as another strain identified as *Bacillus cereus* produced 5-formyluracil, a potent XOI, reported previously by microbial route [18].

A potent XOI, alkalone was identified and purified from the fermentation broth of a marine bacteria, *Agrobacterium aurantiacum* N-81 106[29]. When cultivation conditions of *A. aurantiacum* N-81 106 were changed using a 1000 liter fermenter, alkalone was not produced, and another strong XOI, Hydroxy alkalone, was found to be produced instead of akalone and IC_{50} value was 4.6 µm against XO [30].

A new method was explored to isolate the novel XOI from the fermentation broth of *Lactobacillus rhamnosus* [31] and furthermore 51 novel *Acetobacter* and *Gluconobacter* strains were screened to isolate XOI. Nevertheless, only seven strains have produced the inhibitors showing the more than 30% inhibition. In particular *Acetobacter pasteurianus* strain, AHUO1 has shown 73.6% inhibition [32, 33].

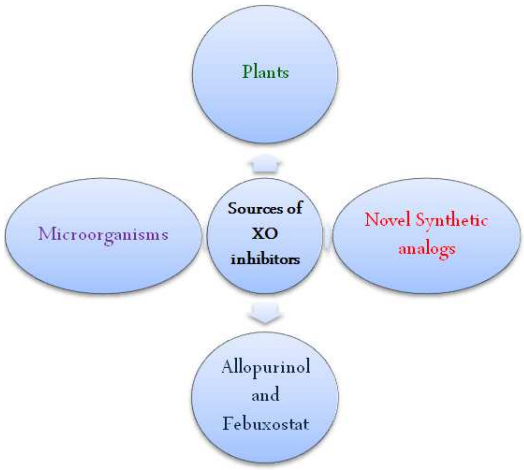

Fig. 1: Various sources of XO inhibitors (Self-designed)

Actinobacteria

Actinobacteria especially *Streptomyces* is a proven source of microbial enzyme inhibitors. A new compound, 5-formyl uracil has been isolated from the culture broth of *Streptomyces* species and has shown strong inhibition against XO [18]. This is the only report on actinobacteria as a source of XOI.

Lichens

Lichens are fungal species which are obligate symbionts in plants. Lichens have long been recognized to contain bioactive compounds. Many natural lichens and cultured lichens have been screened for their biological activities, and several novel compounds were also isolated and identified. The phenolics and oxidative derivatives found in the lichens might be responsible for the inhibition of xanthine oxidase [34, 35]. The natural thallus of *Bulbothrix setschwanensis* was screened for bioactive compounds and found to be composed of mycobiont and photobiont-producing atranorin (a depside) and salazinic acid (a depsidone)[36]. It was reported that the acetone extract of the natural thallus and cultured tissue of *B. setschwanensis* was found to have inhibitory activity on the XO. The IC_{50} value was identified as 44.7 and 52.1µg/ml for cultured tissue and natural thallus respectively [37]. Furthermore, recently 31 species of lichens belong to Graphidaceae were reported for their ability to inhibit XO. Among that *Graphina glaucorufa, G. multistricta, G. salacinilabiata, G. assamensis, G. nakanishiana,* and *Phaeographosis indica* has shown strong inhibition of XO with the lower IC_{50} value of 3-4.8 µg/ml when compared to standard allopurinol having an IC_{50} value of 6.37µg/ml [38].

Endophytic fungi

Over the last two decades, endophytic fungi have been demonstrated to be a rich and reliable source of novel bioactive compounds possessing antimicrobial, anticancer, neuroprotective, insulin-mimetic, anti-oxidant properties that may foster great medicinal or agricultural potentials [39, 40]. Endophytic fungi colonize the plants internally without apparent symptoms of their ubiquitous existence and are increasingly being prospected as underexplored resources of novel bioactive compounds [41]. Exploring endophytic fungi for XO inhibition is a nascent area with very scanty preliminary data [42]. Together medicinal plants, endophytic fungi offer themselves as a relatively reliable source of natural products which could inhibit XO and probably enters the medical field as an antihyperuricemic drug. Fusaruside is a chemically a new cerebroside, characterized from the chloroform-methanol (1:1) extract of *Fusarium* sp. IFB-121, an endophytic fungus in *Quercus variabilis* with an IC_{50} value of 43.8±3.6 µm. In addition to Fusaruside, a known cerebroside isolated from *Fusarium* sp. has shown XO inhibitory activity with an IC_{50} value of 55.5±1.8 µm [43]. As well as phenolic compounds isolated from endophytic *chaetornium* sp residing in *Nerium oleander L* (Apocyanaceae) has exhibited an XO inhibitory activity and the IC_{50} value was found to be 109.8 µg/ml [44].

Recently XOI has been isolated from novel species of *Muscodor*, sterile endophytic fungi. Culture filtrates of 7 species of *Muscodor* isolated from *Cinnamomum* and *Aegle marmelos*. Amongst, the chloroform extract of *M. darjeelingensis* exhibited the maximum XOI with an IC_{50} of 0.54l g/ml which was much lower to allopurinol but higher when compared to febuxostat. Nevertheless, 88% reduction in uric acid production by the extract of *M. darjeelingensis* was similar to allopurinol [45]. Similarly, 19 fungal endophytes were isolated from the medicinal plant *Tinospora cordifolia* and evaluated for their XO inhibitory activity. In the qualitative assay, Out of 19 fungal endophytes, 7 endophytes exhibited>30% XO inhibition, of which isolates #1 TCSTITPLM, #53 TCSTITPLM, #105 TCSTITPLM, and #83 TCSTITPLM were found to exhibit XO inhibition in the range of 38–45%. Ethyl acetate and chloroform extract of #1 TCSTITPLM and #53 TCSTITPLM demonstrated potent XO inhibitory action of 69 and 63% respectively [46]. new culture filtrates of 42 endophytic fungi were screened for XO inhibition in the process of searching for new XOI. The chloroform extract of culture filtrate #1048 AMSTITYEL has exhibited the potential inhibition of XO with an IC_{50} value of 0.61 µg/ml which was better than allopurinol (IC_{50} of 0.937 µg/ml) but lower than febuxostat (IC_{50} of 0.076 µg/ml). The endophyte was identified as *Lasiodiplodia pseudotheobromae* isolated from *Aegle marmelos* [47].

Furthermore, endophytic fungi, *Aspergillus niger* IFB-E003 isolated from *Cyndon dactylon* has screened for XOI. The fraction of the *Aspergillus niger* extract has shown four known compounds naphtha $_{\gamma}$ pyrones such as rubrofusarin B, fonsecinone A, a sperpyrone B, and auraspernone A. Amongst, auraspernone A has shown XO inhibition with an IC_{50} value of 10.9 µmol. Moreover, rubrofusarin B and auraspernone A were also proved as strong co-inhibitors on XO [42].

Mushrooms

Over the decade's mushrooms have been used not only as a source of food, but also remarkable and unexplored source of new biologically active natural products [48]. Mushrooms belong to Basidiomycota of fungi, has received great interest because it contains a large number of biologically active compounds such as polysaccharides, glycoproteins, triterpenes, and antibiotics [49]. Recently mushrooms are being explored as a potential source of XO inhibitors. Flavonoids and polyphenolic crude extracts have been reported to exhibit XO inhibition [50, 51] besides the different flavonoid containing plant species, mushrooms also represents a potential source of such compounds [52, 53]. An unusual compound, 5-methyl-3 (2H)–furanone derivative (inotilione) was isolated from the fruiting body of the mushroom *Inonotus* sp. which has exhibited XO inhibition. The fruiting bodies were subjected to ethanol and chloroform-methanol extraction and purification to isolate the compounds exhibiting XO inhibition [54].

The acetonic, methanolic and hot water extracts from the fruiting bodies of *Pleurotus salmoneoshamineus* and *P. nebrodensis* were reported to have XO inhibition which was found to be increased with increasing concentrations [55, 56]. In a similar way, a new compound was purified from the aqueous extract of *Pleurotus ostreaus* and has exhibited XO inhibitory activity with an IC_{50} value of 0.9 mg/ml [57].

A new species of central European Phellinoid Hymenochaetaceae (*Phellinus sesulato*) have screened for XOI. The *in vitro* antioxidant and XO inhibitory assays demonstrated that most of the selected species possess remarkable antioxidant and XO inhibitory action [58].

A new study was reported in which fermented mushroom water extracts with lactic acid bacteria were exhibited XO inhibition. Fermented mushroom water extracts have increased the free radical scavenging activity, and the antioxidant activity of fermented mushroom extracts was further confirmed by XO inhibition [59]. As well as the above species XO inhibitory activity of aqueous and organic (n-hexane, chloroform, and 50% methanol) extracts of 47 wild-growing mushrooms native to Hungary have been reported. Among the 47 species, Hypholoma fasciculare (IC50= 67.76 ± 11.05 µg/ml), Suillus grevillei (IC50= 13.28 ± 1.58 µg/ml), and Tricholoma populinum (IC50= 85.08 ± 15.02 µg/ml) were exhibited high inhibitory activity [60].

CONCLUSION

The disease burden of hyperuricemia remains a major problem and may be increasing day by day. As described in this article, the rich diversity of microorganisms with their unique characteristics emerged as a potential source for the discovery of XOI. This review is a fine effort to compile and present XOI from microbial sources to encourage further research to use natural microbial XOI in the management and treatment of hyperuricemia and other associated disorders.

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CONFLICT OF INTERESTS

There is no conflict of interest

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