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Exploring glycolate oxidase (GOX) as an antiurolithic drug target: Molecular modeling and *in vitro* inhibitor study

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ABSTRACT

Glycolate oxidase (GOX) is one of the principal enzymes involved in the pathway of oxalate synthesis. It converts glycolate to glyoxylate by oxidation and then glyoxylate is finally converted to oxalate. Therapeutic intervention of GOX in this consequence thus found potential in the treatment of calcium oxalate urolithiasis. In present investigation, we explored GOX in search of potential leads from traditional resources. Molecular modeling of the identified leads, quercetin and kaempherol, was performed by employing Glide 5.5.211 (SchrodingerTM suite). In the absence of pure human glycolate oxidase (hGOX) preparation, *in vitro* experiments were performed on spinach glycolate oxidase (sGOX) as both enzymes possess 57% identity and 76% similarity along with several conserved active site residues in common. We aimed to identify a possible mechanism of action for the anti-GOX leads from *Tribuls terrestris*, which can be attributed to anti-urolithic drug development. This study found promising in development of future GOX inhibitory leads.

1. Introduction

Human glycolate oxidase (EC 1.1.3.15, hGOX), a HAOX1 gene product, and member of the well-characterized FMN-dependent α-hydroxy acid oxidase enzyme family which plays a key role in oxalate synthesis leading to deposition of calcium oxalate stones [1,2]. This family also includes *Pseudomonas putida* mandelate dehydrogenase (MDH), the flavin-binding domain of yeast flavocytochrome b2 (FCB2), rat long chain hydroxy acid oxidase (LCHAO), and spinach glycolate oxidase (sGOX). Human glycolate oxidase (hGOX) in abnormal condition catalyzes the FMN-dependent oxidation of glycolate to oxalate, a key metabolite leading to increased excretion of oxalate which results in to the deposition of kidney stones. Hence, GOX attracts attention of researchers as a potential target in the management of excess oxalate synthesis so as to save life of frequent kidney stone formers due to the genetic aberrations [3,4].

In plants, sGOX regulates a key step during photorespiration, the oxidation of glycolate to glyoxylate which is subsequently converted to oxalate. sGOX is the extensively studied glycolate oxidase for activity and inhibition with its known crystal structure [5]. The unique feature of human and spinach GOX for their affinity to identical substrates made us possible to extrapolate their results with each other. Both of these enzymes possess several conserved active site residues; suggestive of a common mechanism for the oxidation of the substrate and the reduction of the flavin ring [6–10]. Therefore on the ethical background in the absence of pure human enzyme preparations, utilization of spinach GOX will possibly benefit in the discovery of a new drug for the management of kidney stones in humans.

Tribulus terrestris Linn (*Tt*), belonging to the Zygophyllaceae family, traditionally used for various disease ailments both in Indian and Chinese systems of medicine. *Tt* crude extracts have been explored for chemo preventive and chemotherapeutic properties in many human disorders including kidney stone, reported elsewhere. Epidemiological studies are also convincing and shown positive correlations. The major constituents of *Tt* are steroidal saponins [11–28] and flavonoids [29,30]. Although the crude extracts of *Tt* was found to be effective in kidney stone management, a detailed study of its contents for therapeutic intervention of kidney stone is not sufficiently explored yet. This paper focuses on the molecular modeling and docking studies of common flavonoids from *Tt*, specific for sGOX, which are isolated employing bioassays. The investigation of enzyme–ligand interactions has revealed a possible mechanism of action of leads in the management of calcium oxalate stones.

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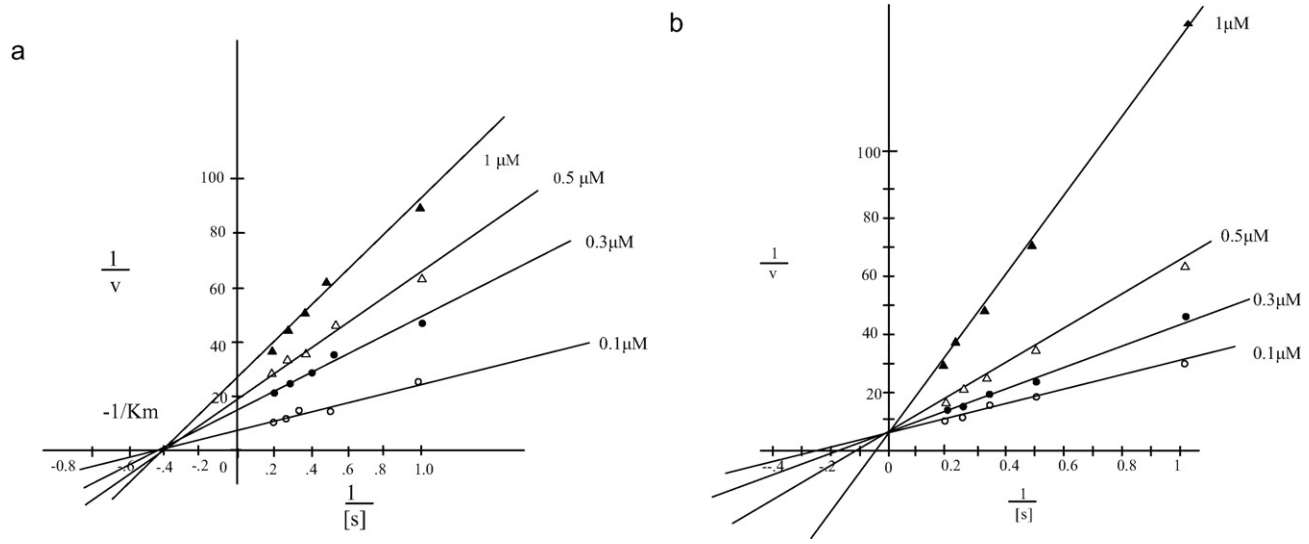

Fig. 1. (a) Lineweaver–Burk plot of inhibition of glycolate oxidase by quercetin. (b) Lineweaver–Burk plot of inhibition of glycolate oxidase by kaempherol.

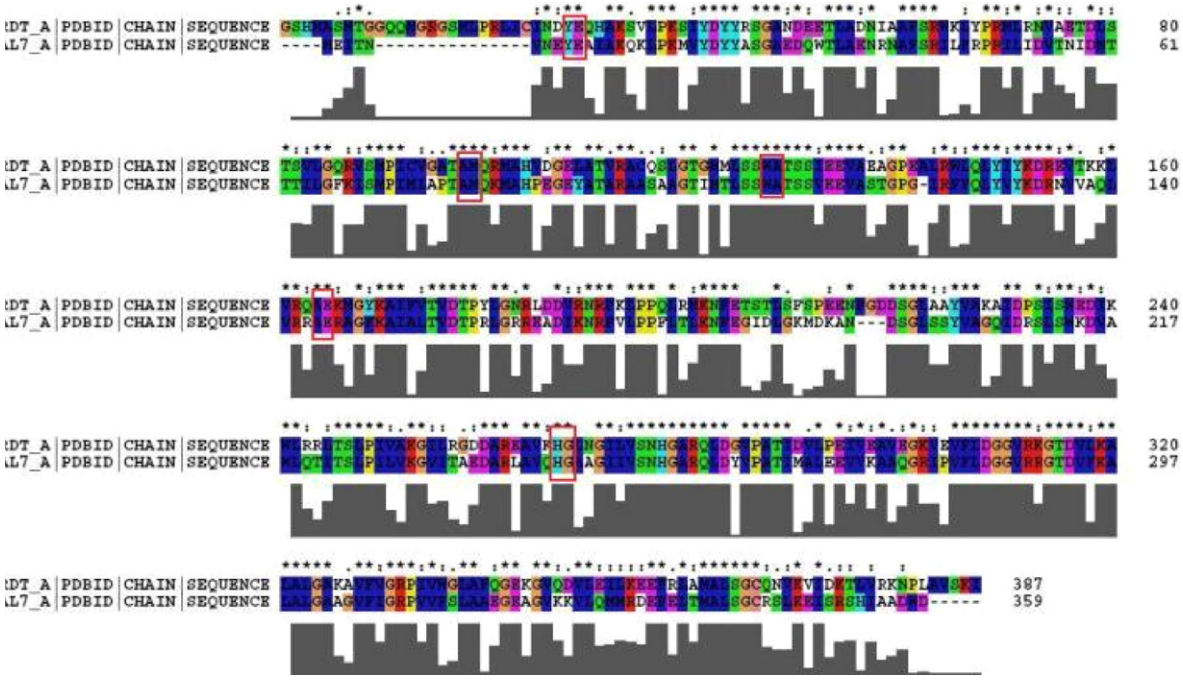

Fig. 2. Multiple sequence alignment of human and spinach GOX in CLUSTAL 2.0.12. (*) Identical amino acids; (.) nearly similar amino acids.

2. Materials and methods

2.1. In vitro GOX inhibition

Glycolate oxidase reaction was coupled with the peroxidase reaction and assayed at 30 °C by monitoring H_2O_2 production. The assay conditions were 1 mM, pH 7.8, Tris–HCl containing 5 µM glycolate, 1 µM antipyrine, 0.5 U horseradish peroxidase, 2 µM phenol, and 0.1 µM FMN in a final volume of 3.0 ml. Assays were initiated by the addition of glycolate and the absorbance increase at 520 nm was then monitored for 2 min. One unit of glycolate oxidase activity was defined as the amount of enzyme required to produce 1 µmol H_2O_2 per min [31].

Bioassay guided fractionation of active crude methanolic extract was achieved from fruits of *T. terrestris* L. and most active organic fraction was fractionated using Sephadex gel chromatography with chloroform as mobile phase. sGOX inhibition by isolated flavonoids from active extracts at variable concentration, 0.1, 0.3, 0.5 and

Table 1
In vitro inhibition values of the compounds with spinach GOX.

Compound	Type of inhibition	K_i value (µM)	IC_{50} (µM)	% Inhibition value
1. Quercetin	Non-competitive	0.56	0.22	58%
2. Kaempherol	Competitive	0.37	0.3	61%