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There are an estimated 300-500 million cases of malaria and up to 3 million people die from this disease annually [1]. The malarial parasite is totally dependent on *de novo* pyrimidine biosynthesis, which is consisted of six reaction steps [2], [3], [4]. In the final two steps, uridine 5'-monophosphate (UMP) requires the addition of a ribose phosphate moiety from 5-phosphoribosyl-1-pyrophosphate to orotate by orotate phosphoribosyltransferase (EC 2.4.2.10, OPRT) to form orotidine 5'-monophosphate (OMP) and pyrophosphate (PP_i), and the subsequently decarboxylation of OMP to form UMP, by OMP decarboxylase (EC 4.1.1.23, OMPDC). Inhibitors of the *de novo* pathway have strong antimalarial activity for *in vitro P. falciparum* growth [5], [6], [7], [8].

In usual *in-silico* technique, the chemical compound is chosen as a candidate in order of docking score. However, hit rate is low *in vivo* and *vitro*. Fukunishi and Nakamura developed Multiple Target Screening (MTS) and Docking Score Index (DSI), both of which use the matrix of ΔG containing several million compounds and several hundreds of protein structures to select the candidates that the most strongly with the target protein among the protein list in the matrix [9], [10].

On the other hand, the DSI method was developed as a method for screening similar compounds by using the matrix like MTS method. The MTS method need the three-dimensional structure of the target protein, however, the DSI method does not require the structure, but the matrix for screening of the similar compound. Generally, the similarity of the chemical compound is shown in the index of parameters in physical chemistry like hydrophobicity and molecular weight.

In this study, we reported a novel compound, 4-(2-hydroxy-4-methoxyphenyl)-4-oxobutanoic acid, exhibited a competitive inhibition with an IC_{50} value of 170 μM, was found by *in silico* assay using multiple target screening MTS, DSI and MM-PBSA [11], in addition to *in vitro* assay using SPR analyses and inhibition assay, furthermore the binding motif of the inhibitor was confirmed by the X-ray structural analysis.

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Functional characterization of UDP-glucose-4-epimerase from *Aspergillus nidulans*

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Pathogenic fungi, namely *Aspergillus* spp., have gained notoriety in recent decades for posing an increased threat to human health[1]. Many challenges exist for treating fungal pathogens in human hosts due to the close evolutionary relationship between the eukaryotic systems[2]. In addition to the limited number of viable drug targets within fungi, most treatments are inhibited by either host toxicity or reduced potency due to the recent emergence of fungal drug resistance[3]. Antifungal drugs which block cell-wall synthesis are believed to be the most promising candidates for clinical treatment[4].

In this context, we are interested in the catalytic pathway of *Aspergillus nidulans* UDP-glucose-4-epimerase (GALE) which produces precursor building blocks for fungal lipopolysaccharide (LPS) biosynthesis. Specifically, GALE is responsible for reversibly inverting the 4'-hydroxyl configuration of UDP-glucose to form UDP-galactose. Given that GALE exhibits interspecies variation, targetable differences between GALE of the host and pathogen can be exploited for rational drug design. In order to identify such differences, detailed structural characterization of *A. nidulans* GALE is required. Ultimately, our goal is to elucidate the structure-function relationship responsible for galactose metabolism in *A. nidulans* GALE.

Crystals of *A. nidulans* GALE grown in the presence of UDP-Galactose via microbatch were diffracted to 2.4 Å resolution at the Canadian Light Source (CLS) 01ID-1 beamline. Examination of the data revealed non-merohedral twinning from which a single lattice was ultimately extracted for data processing. The final space group was *C*2 with unit-cell parameters a = 66.13Å, b = 119.15Å, c = 161.42Å, and β = 98.48°. The structure was solved by molecular replacement with human GALE (PDB code 1hzj) as the template model and subsequently refined through PHENIX. The electron density revealed the presence of NAD(H) co-factor and a UDP-sugar moiety bound within the active site. Overall, the structure is similar to human GALE and exhibits two distinct regions: an N-terminal domain characterized by a modified Rossmann fold of seven strands of parallel β-sheets flanked on either side by α-helices believed to be responsible for co-factor positioning and a C-terminal motif composed of six β-strands and five α-helices postulated for UDP-sugar binding. The characteristic YXXXK motif of the short chain dehydrogenase-reductase superfamily is contained within the N-terminal domain.

Inspection of the *A. nidulans* structure revealed nearly identical positioning of equivalent active-site residues which were shown to be responsible for NAD(H) anchoring and UDP-sugar binding in human GALE. Site-directed mutagenesis and kinetic studies on active-site residues indicate the *A. nidulans* mechanism is similar to that observed in the human form. Interestingly, while we have found *A. nidulans* GALE to be active with larger N-acetyl substrate derivatives, mutations which should inhibit the conversion of these substrates, based on previous biochemical studies of *E. coli* GALE, do not follow the expected trend.

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Structural study of the intertwined dimers of SH3 domains: biological implications of the domain swapping

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