Investigating the inhibitory effect of azo-dyes on the activity of chorismate synthases from various organisms

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Introduction

In the 1950s, Bernhard Davis and David Sprinson discovered a biosynthetic route named shikimate pathway to the aromatic amino acids phenylalanine, tryptophan, and tyrosine. As this metabolic pathway is only present in prokaryotes, fungi, and plants, mammals must obtain aromatic amino acids from their diet.

The inhibition of this pathway can eventually lead to cell death because each step of the shikimate pathway comprises an essential reaction in chorismate synthesis, which cannot be bypassed by any alternative enzyme. The severe consequences for prokaryotes, fungi, and plants when their shikimate pathway is inhibited and the absence of this pathway in mammals make this metabolic pathway a promising target for the development of antibacterial agents and herbicides.

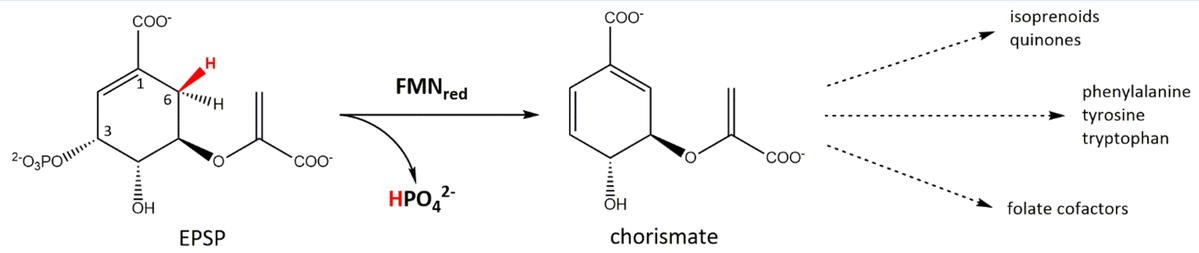

Figure 1: Schematic representation of the seventh and last step of the shikimate pathway. The reaction is catalyzed by chorismate synthase (CS) and includes the conversion of EPSP (5-enolpyruvylshikimate-3-phosphate) to produce the final product of the pathway, chorismate. Chorismate is the starting material for the synthesis of a plethora of aromatic secondary metabolites.

Inhibitors

Chorismate synthase (CS) inhibitors were selected using a combination of virtual screening and molecular dynamics performed by Seixas and co-workers (1, 2). By designing and finding new inhibitory compounds, the extremely positively charged binding pocket of CS was considered. The active site of the chorismate synthase from *Paracoccidioides brasiliensis* (*Pb*CS) was used to illustrate the interaction between the inhibitory compound and the enzyme (Figure 2B).

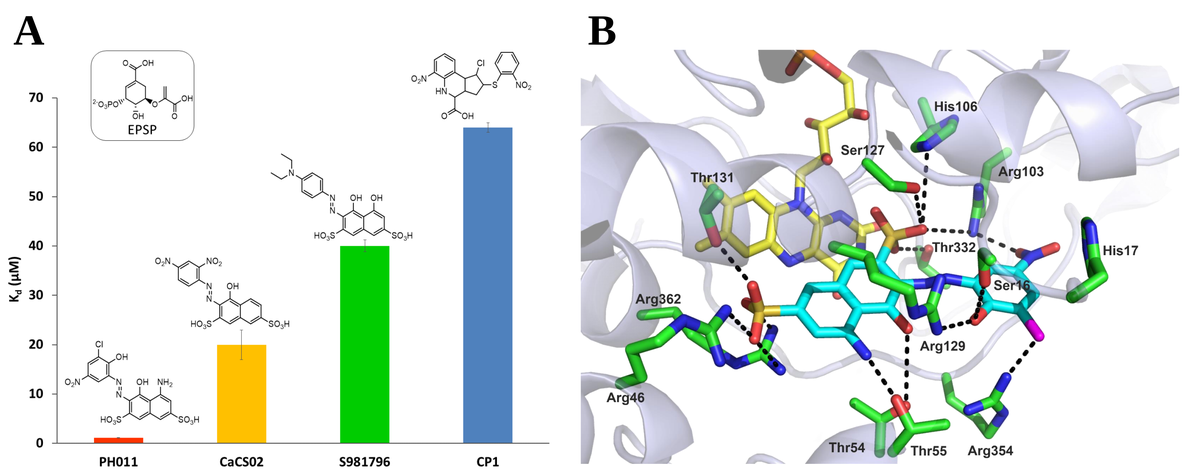

Figure 2: K_D-values of four important inhibitory compounds and their chemical structures (the structure of the 5-enolpyruvylshikimate-3-phosphate (inset) which is the starting material of the CS reaction, the molecules selected by virtual screening (CaCS02 and CP1), and the azo-dyes PH011 and S981796) **(A)**. The inhibitory compound PH011 docked into the active site of *Pb*CS with all residues involved illustrated as green sticks **(B)**.

Different binding behavior?

For the development of new inhibitory compounds, the investigation of the active site of the enzyme is crucial. In principle, the active site of all investigated chorismate synthases is assembled by many invariant arginine residues (Figure 2B). However, structural studies of the active site from different CSs showed one arginine residue that differs, suggesting differences in how the substrate is bound to the active site between different CSs. To get a better insight into the binding mode, it will be necessary to obtain structural information of more CS-inhibitor complexes.

Results

Various methods were used to investigate the most promising inhibitory compound PH011. Among other characteristics, the dissociation constant (1.1 µM) and IC_{50} value (10µM) of *Pb*CS were determined by using a binding and inhibition assay, respectively.

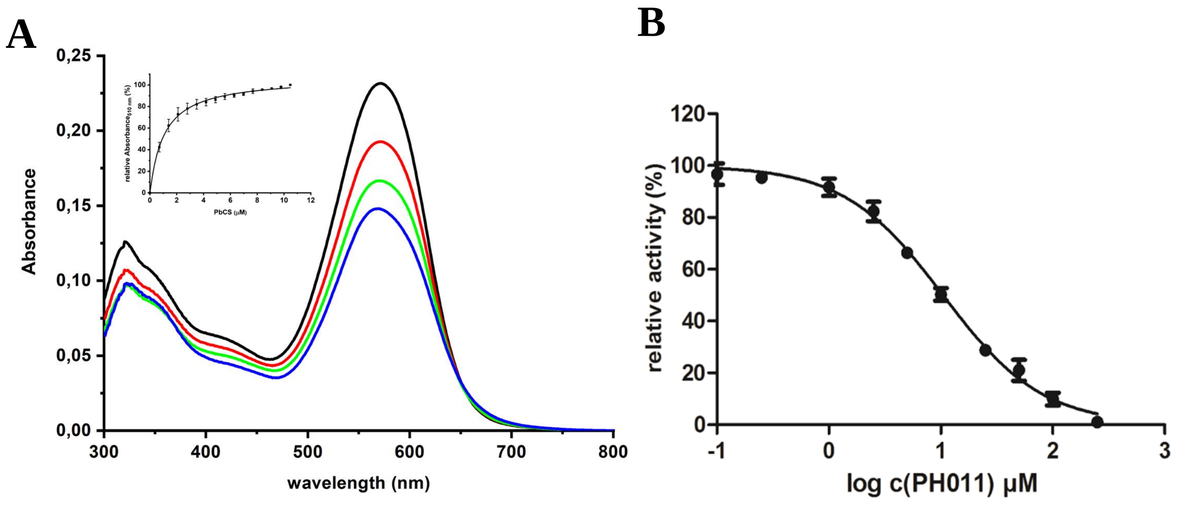

Figure 3: Determination of the binding affinity of the inhibitory compound PH011 toward *Pb*CS; UV-Vis absorption spectra und plot (inset) of the binding assay **(A)**. Activity assay of *Pb*CS with PH011 confirmed the inhibition of the enzymatic activity of the chorismate synthase **(B)**.

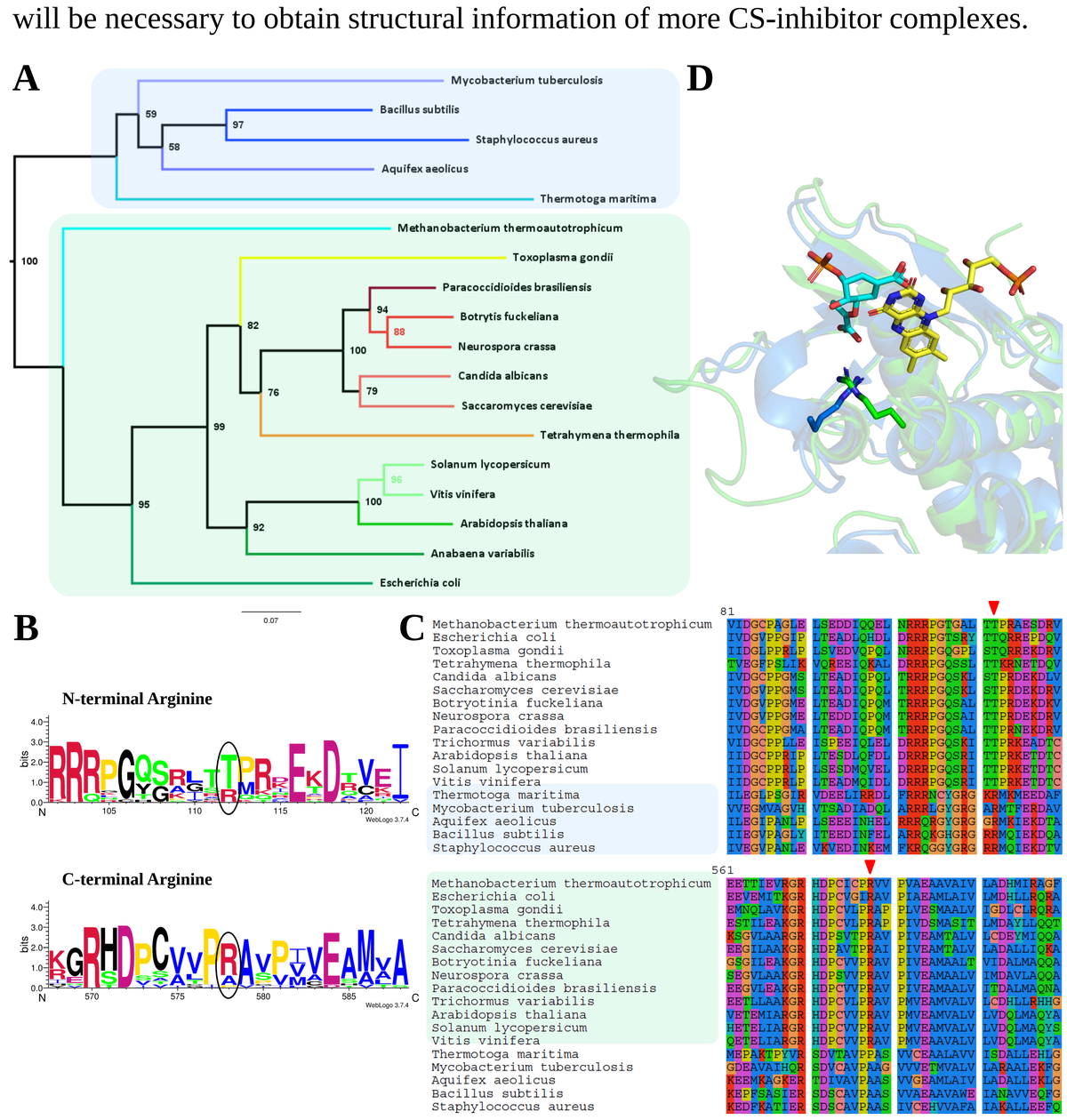

Figure 4: Phylogenetic tree of chorismate synthases from various organisms; the branches are highlighted in blue (N-terminal arginine) and green (C-terminal arginine) **(A)**. Sequence logos of the N- and C-terminus, with the respective arginine highlighted **(B)**. Multiple sequence alignments of all investigated CSs; a red arrow indicates the arginine residue either on the N- or C-terminus **(C)**. Schematic presentation of the active site of *Mycobacterium tuberculosis* (blue) and *Saccharomyces cerevisiae* (green) with the relevant arginine residue in corresponding colors **(D)**.

Outlook

- Crystallization of chorismate synthases from these subclasses to identify/confirm different binding modes.
- Structure guided synthesis and analysis of new inhibitory compounds to improve binding to CS.

References: (1) Rodrigues-Vendramini et al. (2019) American Society for Microbiology, (2) Bueno et al. (2019) Future Microbiology