Development of Inhibitors of PhzE – A New Target for Antibiotic Intervention

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Introduction

Phenazines are mainly produced by *Steptomyces* and *Pseudomonas* as antibiotics and virulence factors and are therefore essential for bacterial survival. The enzyme PhzE converts chorismic acid to 2-Amino-2-deoxyisochorismate (ADIC) in the first step of the biosynthesis of phenazines. Considering the fact that *Streptomyces* and *Pseudomonas* are ubiquitous in medical institutions, there is no doubt that studies towards potent inhibitors are highly relevant.[1] A synthetic approach is conducted in order to provide inhibitors for binding studies. The synthesis of chorismic acid derivatives remains a big challenge, since they are thermodynamically unstable and include stereogenic centers. A successful synthesis of inhibitors could be a big step towards antibiotic intervention.

Inhibitor Design

The enzyme PhzE is related to anthranilate synthase (AS) as it contains two enzymatic activities, encoded in a glutaminase type-I domain (GATase1) to generate ammonia from glutamine and a second domain of the menaquinone, siderophore, tryptophan (MST) type that utilizes the incoming NH_3 to modify chorismate to ADIC (Fig. 1 and 2).[2]

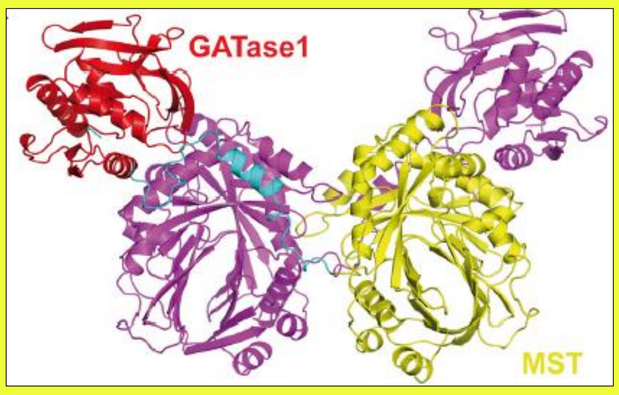


Figure 1. Active domains of PhzE.

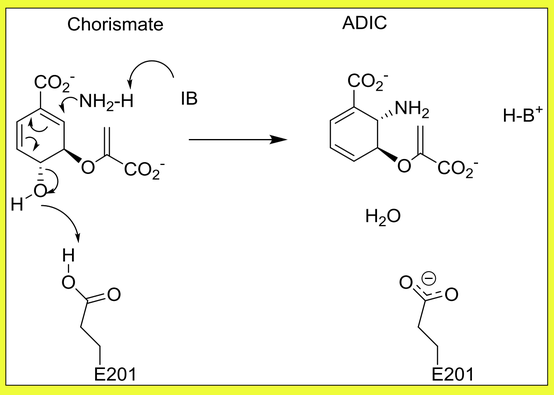


Figure 2. Mechanism of the conversion from chorismate to ADIC

Our aim is to design an inhibitor which binds to the active site. Therefore the proposed structures are similiar to the natural substrate in its electronic and steric properties. Importantly, the inhibitor should not get converted by the enzyme and block the active site. We propose substrates **E-1** to **E-4** for the inhibition of PhzE (Fig. 3).

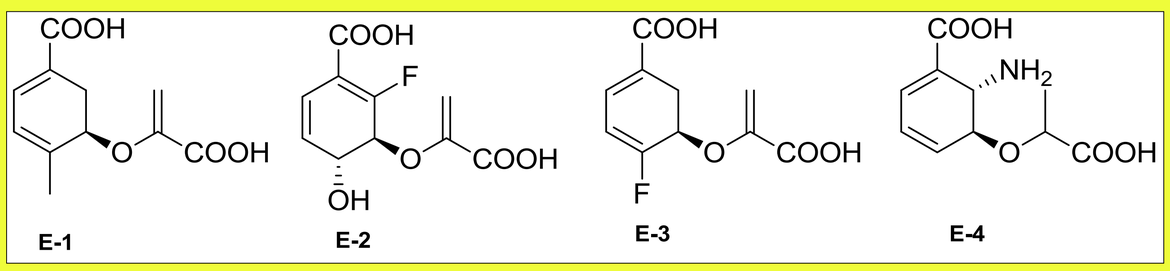
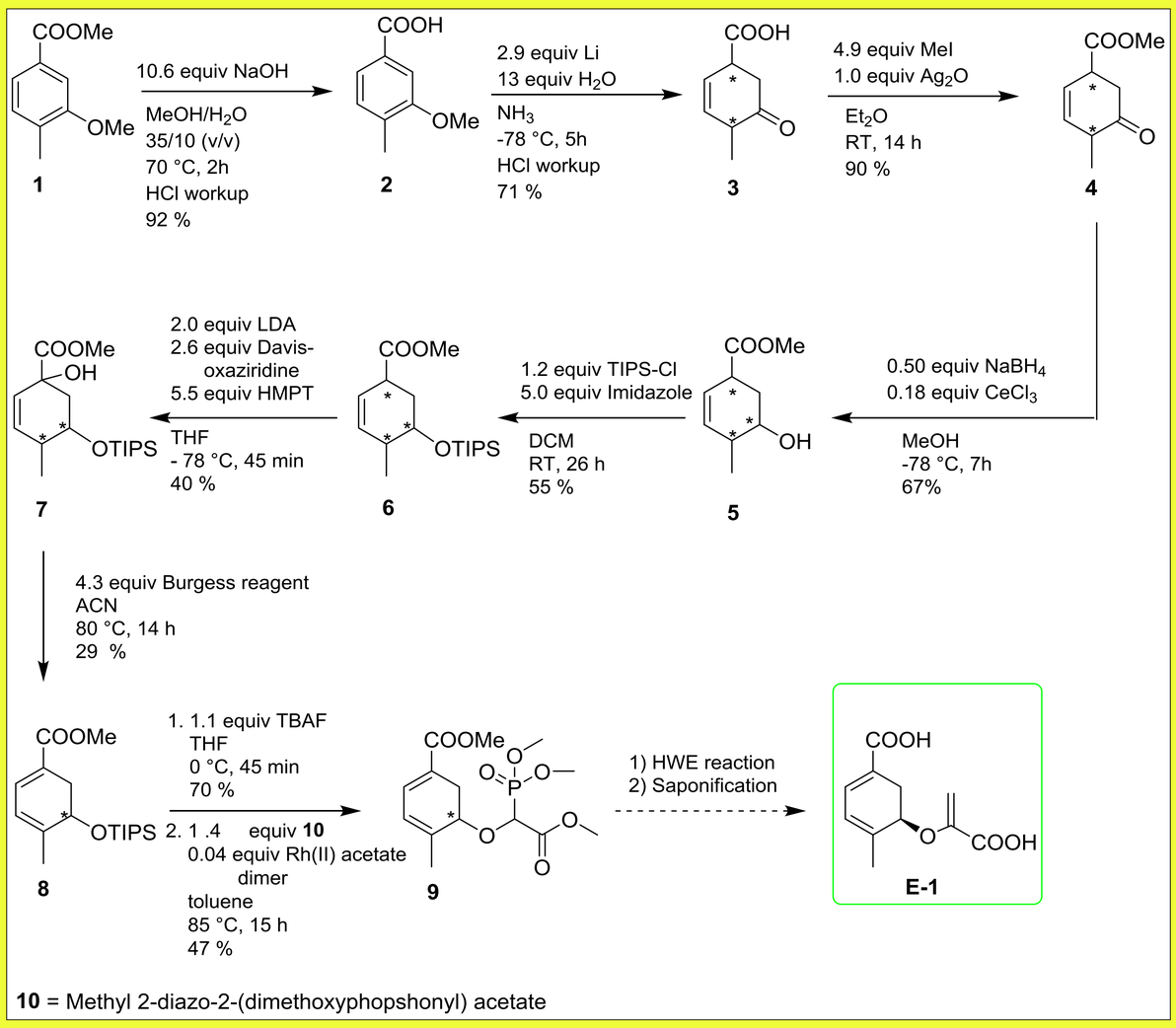


Figure 3. Proposed inhibitors of PhzE.

Results and Discussion

A straightforward approach for the synthesis of **E-1** was envisioned. Starting from inexpensive Methyl 3-methoxy-4-methylbenzoate **1** (Scheme 1), saponification followed by Birch reduction and aqueous hydrolysis afforded compound **3**. The thermodynamical instable intermediate **3** was immediately esterified and reduced, followed by protection of the resulting alcohol to produce **6**. A screening of several double-bond formation methods identified the introduction of an α-hydroxyl group followed by elimination of water via the Burgess reagent as the most appropriate method to introduce unsaturation. The elimination of water from **7**, followed by a spontaneous rearrangement yielded compound **8**, which was converted to **9** by deprotection of the alcohol and subsequent Rh(II)acetate catalyzed coupling. **9** should be converted to **E-1** by a Horner-Wadsworth-Emmons reaction and saponification.

E-1 is a key compound as its synthesis serves as a template for **E-3** and some of its intermediates will be tested for their enzyme activity, too. Furthermore, the synthetic pathway reveals insights into the extraordinary properties of such compounds.



*Scheme 1. Linear synthesis of **E-1**.*

Summary and Outlook

A straightforward synthesis for the key compound **9** was developed, which involves the handling of some very instable intermediates. Compound **9** serves as an advanced intermediate for the synthesis of inhibitor **E-1**. Compound **E-1** is a key compound as its synthesis serves as a template for **E-3** and some of its intermediates of the synthesis will be tested for their enzyme activity. Binding and crystallographic studies towards the enzyme PhzE will be performed with **E-1**- **E-4**. An inhibitor of PhzE would knock out the biosynthesis of phenazines, which would reduce the virulence of several pathogenic bacteria. The study of substrates **E-1** to **E-4** as potent inhibitors fits into the literature as similar compounds proved to be good inhibitors for the PhzE-related enzyme anthranilate synthase (AS).[3]

Acknowledgements

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References:

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