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Cinnamic acid derivatives as inhibitors for chorismatases and isochorismatases

Florian Hubrich, Silja Mordhorst, Jennifer N. Andexer *

Pharmaceutical and Medicinal Chemistry, Institute of Pharmaceutical Sciences, University of Freiburg, Albertstr. 25, 79104 Freiburg, Germany

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ABSTRACT

Chorismatases and isochorismatases catalyse the hydrolysis of chorismate or isochorismate leading to unsaturated cyclohexenoic acid derivatives. Based on simplification of the physiological substrates, two cinnamic acid-derived compounds, differing in the saturation of the side chain, were developed. In contrast to earlier inhibitor studies, the compounds described here do not have an ether bond and therefore can be synthesised very easily in one or two steps without the need for protective groups. Both substances demonstrate inhibition of the isochorismatase EntB from *Escherichia coli* and the chorismatases FkbO and Hyg5 from *Streptomyces*. For chorismatases, the unsaturated compound shows IC_{50} values in the millimolar range, while the saturated compound is the better inhibitor with IC_{50} values in the micromolar/low millimolar range; for the isochorismatase tested both compounds inhibit in the micromolar range. Further, an analysis of the apparent K_m values for FkbO and EntB was performed, showing that both inhibitors act in a competitive manner. Due to the ease of modifying these new inhibitors they are a suitable starting point for exploring further functionalised derivatives.

Chorismic acid is a central branching point in the metabolism of bacteria, plants and fungi. A range of enzyme classes catalyse the conversion of chorismic acid into different small molecules, including chorismate mutase (Claisen condensation to prephenic acid), chorismate lyase (cleavage of the pyruvate moiety resulting in *para*-hydroxybenzoic acid), isochorismate synthase (isomerisation to isochorismic acid) and salicylate synthase (resulting in *ortho*-hydroxybenzoic acid). The latter two belong to a group of chorismate-utilising enzymes that are structurally related and employ very similar mechanisms.[1–3] The products of chorismate-utilising enzymes may be further converted to aromatic amino acids and quinones or used as building blocks in the biosynthetic pathways of more complex natural products like siderophores.[4,5]

Due to the absence of the shikimate pathway in mammals, its enzymes have been exploited as a target for many inhibitor studies leading to herbicides[6] or antibiotic agents.[7] Among these compounds are many chorismate analogues serving as competitive inhibitors; for chorismate mutase, a range of adamantane-derived compounds mimicking the transition state during the rearrangement to prephenate have been described.[8,9]

The isochorismate synthase family (isochorismate synthase, salicylate synthase, anthranilate synthase, amino-deoxychorismate synthase) belongs to one of the most intensely studied groups of these enzymes. Starting from dihydrochorismate-derived transition state models,[3] the group of Abell explored many chorismate derivatives ranging from aromatic compounds to carboxypyridine-derived analogues.[10–13] In another study, a library of compounds linked to a solid support was tested on aminodeoxychorismate synthase and other enzymes of the isochorismate synthase family.[14] Further, chorismic acid derivatives have been shown to be substrates for a range of enzymes, among them isochorismatases[15] and the 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase MenD from the menaquinone pathway.[16]

Recently, a new enzyme family using chorismic acid as substrate has been described: chorismatases (Fig. 1) catalyse the hydrolysis of chorismic acid into pyruvic acid and either 3,4-dihydroxycyclohexa-1,5-dienoic acid (3,4-CHD, FkbO-type) or 3-hydroxybenzoic acid (3-HBA, Hyg5-type). The chorismatases characterised so far provide the starting units for polyketide natural products like ascomycin or tacrolimus; their involvement in the biosynthetic pathways of other natural products like brasilicardin has been proposed and the function in of Bra8 (the brasilicardin chorismatase) has been shown in vivo.[17] For xanthomonadin, a chorismatase in the biosynthetic gene cluster has been suggested as well,[17] and now been proven by studies of Zhou et al.[18] The molecular basis of enzymatic chorismatase hydrolysis has not been described yet and the enzymes do not show significant sequence similarity to other chorismate-utilising enzymes.

For isochorismatases a strong side activity for chorismic acid utilisation has been described in various studies.[15,19] The natural substrate of these enzymes is isochorismic acid, which differs from chorismic acid only in the position of a single hydroxyl group

* Corresponding author. Tel.: +49 761 203 67398; fax: +49 203 761 6351.
E-mail address: jennifer.andexer@pharmazie.uni-freiburg.de (J.N. Andexer).

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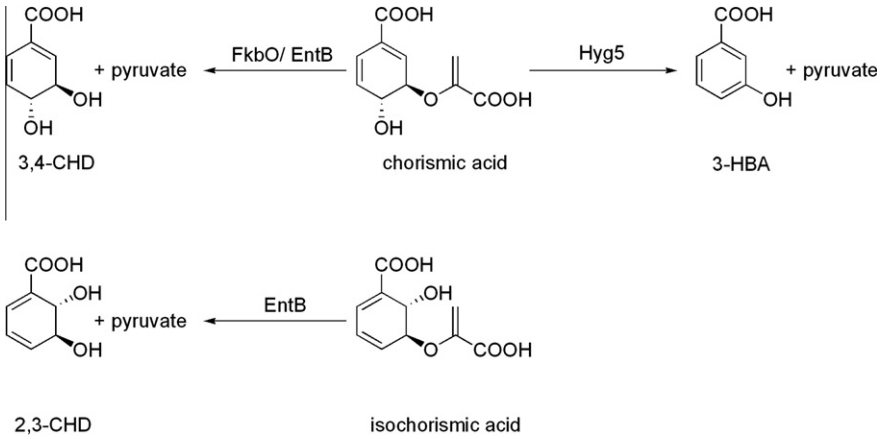

Figure 1. Reactions catalysed by chorismatases and isochorismatases. Chorismatases hydrolyse chorismic acid to pyruvate and 3,4-CHD (FkbO-type) or 3-HBA (Hyg5-type). The physiological substrate for the isochorismatase EntB is isochorismic acid (leading to 2,3-CHD); with chorismic acid it exhibits a FkbO-type side activity.

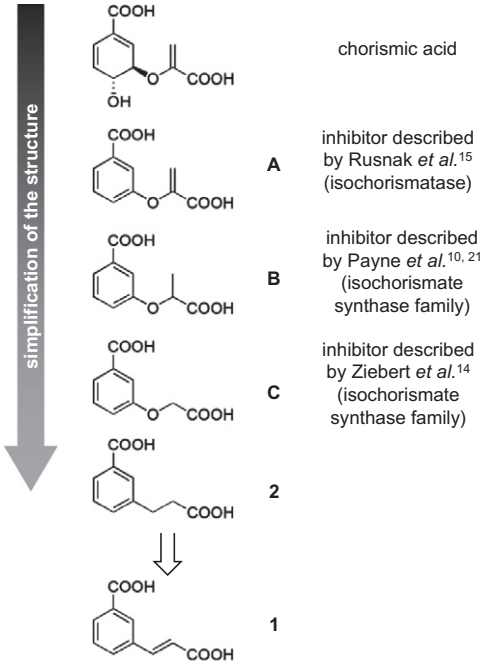

Figure 2. Chorismate analogues as competitive inhibitors for chorismate-utilising enzymes. The compounds are ordered corresponding to their structural complexity.

(Fig. 1). There is no significant sequence homology between isochorismatases and chorismatases (<10% similarity on amino acid basis);[15,19] therefore it is not possible to predict a model structure for chorismatases based on the known isochorismatase structures.[20]

During our efforts to elucidate the three-dimensional structure and the catalytic mechanism of the chorismatases FkbO and Hyg5 we looked for a suitable inhibitor as a helpful additive for crystallisation and to determine the active site and binding mode. Figure 2 gives an overview over chorismate analogues described as competitive inhibitors for different chorismate-utilising enzymes. Compound **A**[15] has been tested with isochorismatases, whereas **B**[10,21] and **C**[14] have been found in studies with enzymes from the isochorismate synthase family. Starting from these compounds we aimed at simplifying this structure further, leading to cinnamic acid derivatives that can be produced in few steps without the need for protective group chemistry.

Compared to a C–O ether bond a C–C bond shows similar angles, bond lengths, hybridisation state and conformational flexibility. One main difference is the ability of the ether oxygen to form hydrogen bonds. However, based on the structures described for isochorismatases, the main stabilisation of the substrate in the active site is thought to be accomplished by hydrophobic interactions with the cyclohexadiene ring and hydrogen bonding with the two carboxyl groups. Hence the ether bond of the known inhibitors **A**–**C** should not be required. Based on these considerations we developed the following two target compounds: 3-(2-carboxyvinyl)

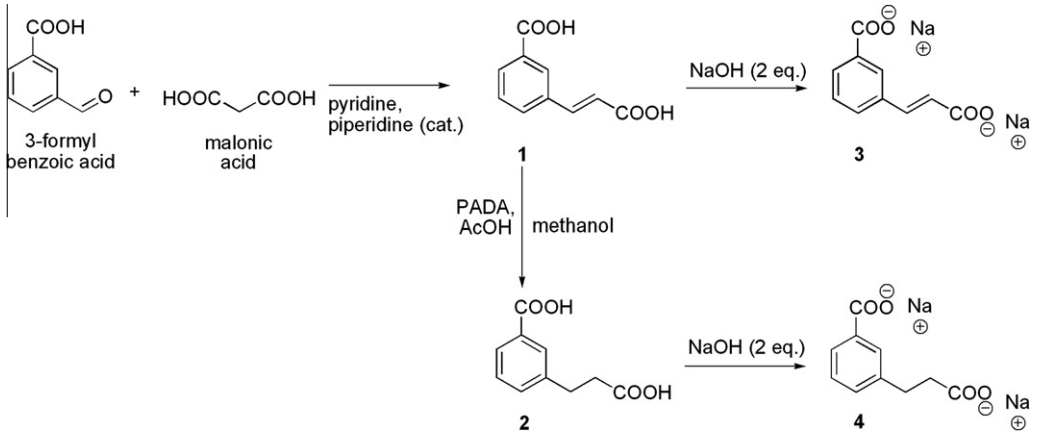

Figure 3. Scheme of synthetic procedures. 3-(2-Carboxyvinyl)benzoic acid (**1**), 3-(2-carboxyethyl)benzoic acid (**2**), and their corresponding sodium salts (**3**, **4**).