S-(−)-Dinitrobiphenic Acid: A Selective Inhibitor of *Escherichia coli* Chorismate Mutase Based on Prephenate Mimicry

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The conversion of chorismate **1** to prephenate **2** in the shikimate biosynthetic pathway is catalyzed by the enzyme chorismate mutase (CM) and represents a rare example of an enzyme-catalyzed pericyclic reaction. X-ray crystallographic analyses of CMs have identified two distinct protein folds that catalyze the rearrangement of **1** to **2**,[1] and provided important clues about enzyme mechanism.[2] The mutase reaction has also served as a model for research into fundamental questions of enzymology such as antibody catalysis,[3] combinatorial mutagenesis and selection,[4] and directed evolution.[5]

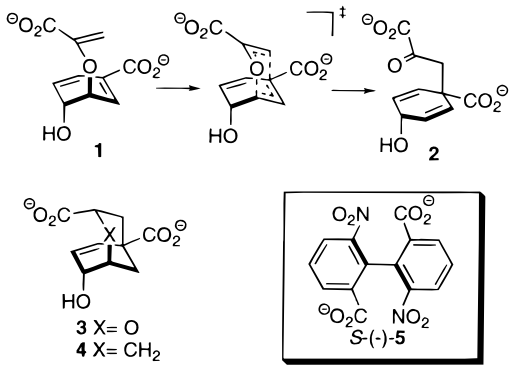
CMs play a central role in the biosynthesis of phenylalanine and tyrosine in microorganisms and plants and are attractive targets for the design of antibiotics and herbicides. Yet limited progress has been made in developing either a highly potent CM inhibitor or one that displays selectivity between mutases. The two most effective CM inhibitors are transition-state mimics **3**[6] and **4**,[7] but neither is selective. Oxabicyclic diacid **3** broadly inhibits CMs from the *Escherichia coli* P- and T-proteins (EcCM), *Bacillus subtilis* (BsCM), and *Saccharomyces cerevisiae* (ScCM) with low-micromolar K_i values.

In the course of exploring approaches to the design of new mutase inhibitors, we have discovered that the *S*-enantiomer of 6,6′-dinitrobiphenic acid [*S*-(−)-DNBA **5**][8] is comparable in potency to **3** as a competitive inhibitor of EcCM ($K_i = 13\ \mu M$), but has no effect on BsCM or ScCM at 600 μM. Here we present kinetic data suggesting that *S*-**5** mimics the reaction product, prephenate **2**, and interacts with the prephenate-binding pocket of EcCM (Scheme 1). A structure for the complex of EcCM with *S*-**5** has been calculated using computer-simulated docking techniques that faithfully reproduce the structures of several known CM-ligand complexes. Additional docking simulations with BsCM indicate that selective inhibition of EcCM by *S*-**5** appears to be the result of size discrimination.

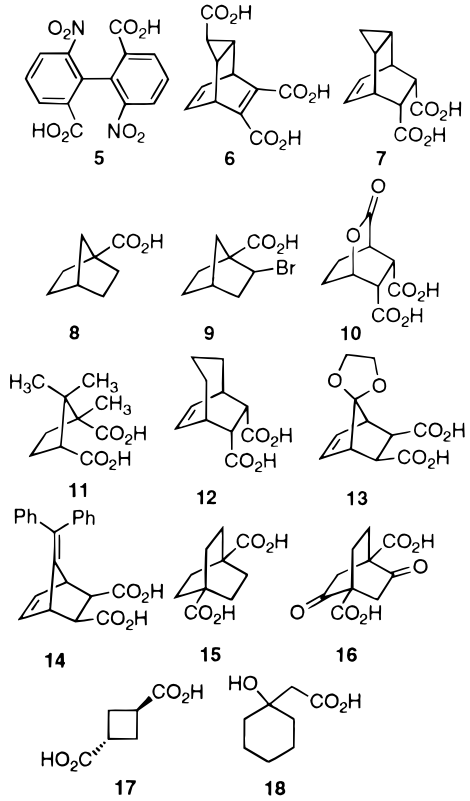
Despite differences in their overall secondary structures and substrate-binding pockets, both EcCM and BsCM require two free carboxylic acid groups to catalyze the rearrangement of chorismate and its analogues.[9,10] Guided by the enzymes' absolute functional group requirement, as well as by crystallographic data and energy-minimized structures of **1** and **2**, a library screening approach was adopted to identify new CM inhibitors. An initial array of racemic, low-molecular weight, mono-, bi-, and tricyclic acids **5**−**18** (Scheme 2) was assembled and screened initially against EcCM in a standard assay [5 min, 37 °C, 50 m*M* tris (pH 7.8), 2.5 m*M* EDTA, 20 m*M* mercaptoethanol, 0.01% BSA, and 333 μM chorismic acid ($K_m = 290\ \mu M$)].[11]

Compounds **6**−**17** had no effect on EcCM at 1 m*M*. Interestingly, the simple hydroxyacid **18** inhibited EcCM with an IC_{50} of 350 μM. However, racemic DNBA **5** exhibited significant levels of inhibitory activity in initial bioassays of EcCM and was subsequently resolved.[10] *S*-(−)-DNBA, whose absolute configuration has been reported,[12] was the more potent inhibitor (IC_{50} =

Scheme 1



Scheme 2



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‡ Section of Biochemistry, Molecular and Cellular Biology.

(1) (a) Lee, A. Y.; Karplus, P. A.; Ganem, B.; Clardy, J. *J. Am. Chem. Soc.* **1995**, *117*, 3627. (b) Chook, Y.-M.; Gray, J. V.; H. Ke, Lipscomb, W. N. *J. Mol. Biol.* **1994**, *240*, 476. (c) Xue, Y.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 10595.
(2) (a) Lee, A. Y.; Clardy, J.; Stewart, J. D.; Ganem, B. *Chem. Biol.* **1995**, *2*, 195. (b) Ganem, B. *Angew Chem., Int. Ed. Engl.* **1996**, *35*, 936.
(3) (a) Jackson, D. Y.; Jacobs, J. W.; Sugasawara, R.; Reich, S. H.; Bartlett, P. A.; Schultz, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 4841. (b) Haynes, M. R.; Stura, E. A.; Hilvert, D.; Wilson, I. A. *Science* **1994**, *263*, 646.
(4) Kast, P.; Asif-Ullah, M.; Jiang, N.; Hilvert, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5043.
(5) MacBeath, G.; Kast, P.; Hilvert, D. *Science* **1998**, *279*, 1958.
(6) Bartlett, P. A.; Nakagawa, Y.; Johnson, C. R.; Reich, S. H.; Luis, A. *J. Org. Chem.* **1988**, *53*, 3195.
(7) Clarke, T.; Stewart, J. D.; Ganem, B. *Tetrahedron* **1990**, *46*, 731.
(8) Ingersoll, A. W.; Little, J. R. *J. Am. Chem. Soc.* **1934**, *56*, 2123.
(9) Pawlak, J. L.; Padykula, R. E.; Kronis, J. D.; Aleksejczyk, R. A.; Berchtold, G. A. *J. Am. Chem. Soc.* **1989**, *111*, 3374.
(10) Galopin, C. C.; Ganem, B. *Bioorg. Med Chem Lett.* **1997**, *7*, 2885.
(11) Gething, M. H.; Davidson, B. E.; Dopheide, T. A. A. *Eur. J. Biochem.* **1976**, *71*, 317.
(12) Newman, P.; Rutkin, P.; Mislow, K. *J. Am. Chem. Soc.* **1958**, *80*, 465.

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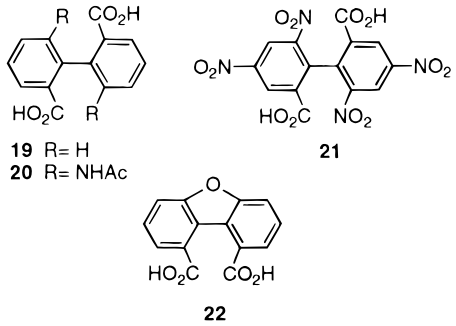
Scheme 3



50 μM; for *R*-DNBA, IC_{50} = 220 μM). Under steady-state conditions, *S*-(−)-DNBA competitively inhibited EcCM with a K_i of 13 μM. In the absence of chorismate, the enthalpy of binding (ΔH = −6.5 kcal/mol) and the enzyme−inhibitor dissociation constant (K_d = 8.3 μM) were determined by isothermal titration calorimetry (ITC).[13] Thus, *S*-**5** was comparable in potency to the oxabicyclic transition-state analogue **3** (K_i = 3 μM against EcCM).[2a] Unlike TS mimics **3** or **4**, however, *S*-**5** did not inhibit BsCM or ScCM at concentrations up to 600 μM.

Other biphenic acid analogues were prepared and assayed, including 2,2′-biphenic acid **19**, bis-acetamide **20**, tetranitrobiphenic acid **21**, and dibenzofuran **22** (Scheme 3). No inhibition was observed with **19**, **20**, or **22**. The strong UV absorbance of **21** interfered with kinetic assays, but ITC measurements confirmed that **21** was bound at least 10-fold more weakly than *S*-**5** to EcCM.

Early studies on the *E. coli* P-protein provided a clue to the selective effect of *S*-DNBA on EcCM. In that bifunctional enzyme, which combines EcCM with a distinct prephenate dehydratase (PDT) activity, prephenate was a competitive product inhibitor of EcCM.[14] Interestingly, K_m for **2** as a substrate for PDT was 16-fold higher than K_i for **2** as an inhibitor of CM,[14] an observation that has largely been overlooked as a rationale for designing mutase inhibitors.

In fact, *S*-**5** also inhibited PDT. Assays under steady-state conditions[15] using a recently reported, fully active, monofunctional PDT domain (PDT22, residues 101−300 of the P-protein)[16] showed that inhibition by *S*-**5** was competitive, with K_i = 300 μM (**2** as substrate, K_m = 710 μM, pH 7.8, 37 °C). Thus, K_i for *S*-**5** as a PDT inhibitor was 23-fold higher than K_i for *S*-**5** as an EcCM inhibitor. The similarity in the relative behavior of **2** and *S*-**5** toward CM and PDT suggested that *S*-**5** mimicked prephenate.

Molecular modeling studies revealed few structural similarities between inhibitors **3** and *S*-**5**. Despite its larger molecular framework, *S*-**5** could be superimposed on **3** with excellent overlap of two carboxylic acid groups. In terms of ligand molecular volume *V*, *S*-**5** (V = 368 Å^3) was substantially larger than the bicyclic transition state mimic **3** (V = 268 Å^3), even taking into account the water molecule complexed with **3** in the EcCM crystal structure (V = 280 Å^3).

In the absence of crystallographic information, the EcCM·*S*-**5** complex was modeled computationally using Quick Explore (QXP), whose algorithms for docking of a flexible ligand with a rigid receptor are based on the method of Monte Carlo perturbation with energy minimization.[17] QXP allows key amino acid residues to move under constraints defined by the program, whereas the remaining coordinates of the protein are fixed. The reliability of QXP was established by successfully docking inhibitor **3** both with EcCM and BsCM and prephenate **2** with BsCM. The solid-state structures of all three complexes have been reported,[1] and the root-mean-square difference between the published structure and the lowest-energy docked structures were 0.33, 0.4, and 0.58 Å, respectively.

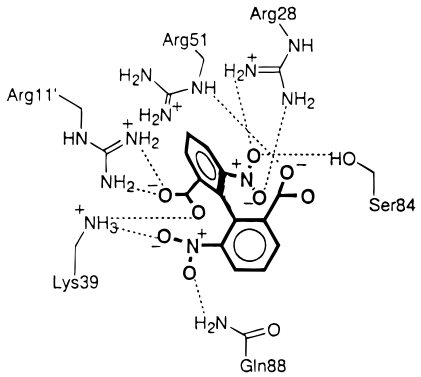


Figure 1. Ligand-docking simulation of *S*-DNBA *S*-**5** in EcCM using QXP. Schematic drawing of hydrogen-bonding and electrostatic interactions of *S*-**5** with relevant side chains of EcCM.

The structure of *S*-**5** docked with the EcCM dimer (Figure 1) revealed strong interactions with many of the residues found in the catalytic site of the mutase. As with transition-state analogue **3**, the two carboxyl groups in the bisected conformation of *S*-**5** formed electrostatic interactions and hydrogen bonds with positively charged Lys39, Arg11′, Arg28, and Arg51 residues (Figure 1b). The two nitro groups of *S*-**5** also interacted favorably with these cationic residues, and formed hydrogen bonds with Ser84 and Gln88 in the active site. Unlike **3**, which employed a molecule of bound water in EcCM to link the three arginines in a network of stabilizing interactions between the two carboxyl groups, *S*-**5** achieved comparable binding using both carboxyl and nitro groups to bridge interactions with cationic residues across its expanded molecular framework. With its smaller dimensions, the BsCM active site was unable to accommodate *S*-**5**. Docking experiments using QXP reproducibly displaced *S*-**5** outside the binding pocket, leaving only one ring of the inhibitor proximal to active site residues.

In conclusion, we have shown that product mimicry constitutes an effective approach to CM inhibitors and deserves more widespread consideration in structure-based drug design strategies. In addition, selective inhibition of chorismate mutases can be achieved by capitalizing on heretofore-overlooked differences in the molecular dimensions of mutase active sites. The nonobvious resemblance between **2** and its mimic *S*-**5** suggests that detailed structural information about other CM−product complexes might be exploited to develop more potent and selective CM inhibitors. The recent report that chorismate synthase and other shikimate pathway enzymes have been detected in certain parasites has heightened interest in this area.[18]

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Supporting Information Available: Lineweaver−Burk plots of kinetic data from enzymatic assays of *S*-**5** and the results of docking **2**, **3**, and *S*-**5** with EcCM and BsCM using QXP (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Titrations were carried out using an Omega titration microcalorimeter from MicroCal, Inc. (Northampton, MA) in 20 mM Tris, pH 8.2, and 0.1 M NaCl using 51 μM EcCM (dimer) at 15 °C.

(14) Duggleby, R. G.; Sneddon, M. K.; Morrison, J. F. *Biochemistry* **1978**, *17*, 1548.

(15) Assays were performed as reported in ref 11.

(16) Zhang, S.; Pohnert, G.; Kongsaeree, P.; Wilson, D. B.; Clardy, J.; Ganem, B. *J. Biol. Chem.* **1998**, *273*, 6248.

(17) McMartin, C.; Bohacek, R. S.; *J. Comput-Aided Mol. Des.* **1997**, *11*, 333, 344.

(18) Roberts, F.; Roberts, C. W.; Johnson, J. J.; Kyle, D. E.; Krell, T.; Coggins, J. R.; Coombs, G. H.; Milhous, W. K.; Tzipori, S.; Ferguson, D. J. P.; Chakrabarti, D.; McLeod, R. *Nature* **1998**, *393*, 801.