Selective Inhibition of Type II Dehydroquinases

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The syntheses of the first inhibitors of the type II dehydroquinase (3-dehydroquinate dehydratase) are described. Dehydroquinase catalyses the reversible conversion of 3-dehydroquinic acid **1** to 3-dehydroshikimic acid **2** on the shikimic acid pathway to aromatic amino acids.[1] The same reaction is also a step on the quinic acid pathway used by fungi to metabolize quinic acid as a carbon source.[2] There are two distinct types of dehydroquinase.[3] Type I dehydroquinases are typified by the *E. coli* enzyme, which is a dimer of subunit M_r 27466,[4] whereas type II dehydroquinases are dodecameric proteins of subunit M_r 12000−18500.[5,6]

The presence of type II dehydroquinase as part of the shikimate pathway in *Mycobacterium tuberculosis*,[7] *Helicobacter pylori*,[8] and *Streptomyces*[9] make it an interesting and novel antibiotic target. However, in designing inhibitors for this enzyme the problem of discrimination from the type I enzyme must be addressed as both types of dehydroquinase are likely to have similar recognition elements in the active site. However, consideration of the two enzyme mechanisms suggests a rational basis for this discrimination. The type I enzyme catalyzes an overall syn dehydration involving loss of the 2-*pro*-*R* hydrogen.[10] The mechanism proceeds through a series of imine and enamine intermediates[11] formed with a conserved active site lysine.[4] In contrast, the mechanism of the type II enzyme does not involve any covalent attachment of the substrate to the enzyme. The elimination has been shown to proceed by an E_1CB mechanism, probably *via* an enolate intermediate **3**,[12] and involves loss of the more acidic 2-*pro*-*S* hydrogen of the substrate, in an anti elimination of water (Scheme 1).[13] The intermediate **3** is characterized by the flattening of the carbocyclic ring and probably involves stronger hydrogen bonding to the enolate oxygen in the transition state. We have exploited these two features separately in the design of the first inhibitors of type II dehydroquinases.

Our first target compound was 2,3-anhydroquinic acid **10**. This is structurally similar to the substrate **1** but lacks the carbonyl group necessary to form an imine with the type I enzyme. In addition, the C2−C3 double bond mimics the flattening of the ring in the enolate intermediate **3**. To assess the contribution that this subtle conformational restriction makes to binding, the reduced compound 3-deoxyquinic acid **11** was also prepared.

Both **10** and **11** were synthesized from (−)-quinic acid **4** (Scheme 2). Treatment of quinic acid with benzaldehyde in hot acidic toluene afforded an epimeric mixture of 3,4-*O*-benzylidenequinides **5** (75%),[14] which were readily converted to the *trans*-bromobenzoate **6** (86%) and thence to the silyl ether **7** (83%) using previously reported protocols.[15] Fluoride-induced desilylation of **7** yielded allylic alcohol **8** (88%), which upon treatment with catalytic methoxide underwent lactone methanolysis with concomitant debenzoylation to afford ester **9** (96%). Saponification of ester **9** followed by ion exchange afforded the desired acid **10** (99%), which upon hydrogenation yielded 3-deoxyquinic acid **11** (97%).

Our second design strategy was to look for extra binding affinity in the carbonyl binding pocket of the type II dehydroquinases, where it is assumed that stabilization is provided for the formation of the enolate intermediate **3**. The target compound was the simple oxime **19**. As a control we made the compound **13** with the *exo*-methylene group (Scheme 3). Three-step deprotection of the known[16] exocyclic olefin **12** proceeded smoothly to afford the desired acid **13**. The oxime **19** was prepared from the known lactone **14**.[14] Methoxide-catalyzed lactone cleavage occurred with concomitant silyl migration to afford a chromatographically separable mixture of ketoesters **15** (35%) and **16** (49%). These were readily converted to their corresponding oximes **17** (84%) and **18** (92%) (single isomers in each case), formulated as the *E* isomers both on steric grounds and on the basis of downfield shifts of the equatorial hydrogens at C-2 due to the neighboring oxygen atom of the oxime moiety. Fluoride-induced desilylation of **18** followed by saponification afforded the oxime **19** (isolated as its sodium salt).

The four acids **10**, **11**, **13**, and **19** were assayed in the presence of dehydroquinate for their inhibitory properties against type I dehydroquinase (from *Salmonella typhimurium*) and against type II dehydroquinases (from *Aspergillus nidulans*, *M. tuberculosis*, and *Streptomyces coelicolor*).[17] The inhibition data are summarized in Table 1. All of the inhibitors showed some level of competitive reversible inhibition of both type I and II dehydroquinases. The inhibitors **10** and **19** were clearly selective for type II dehydroquinases and exhibited unexpected discrimination between different type II enzymes.

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(17) All assays were performed at 25 °C in quartz cuvettes (total reaction volume 1 mL) buffered at pH 7.0 (type I, 50 mM phosphate; type II, 50 mM Tris−HCl) and were monitored spectrophotometrically at 234 nm for the appearance of dehydroshikimate.

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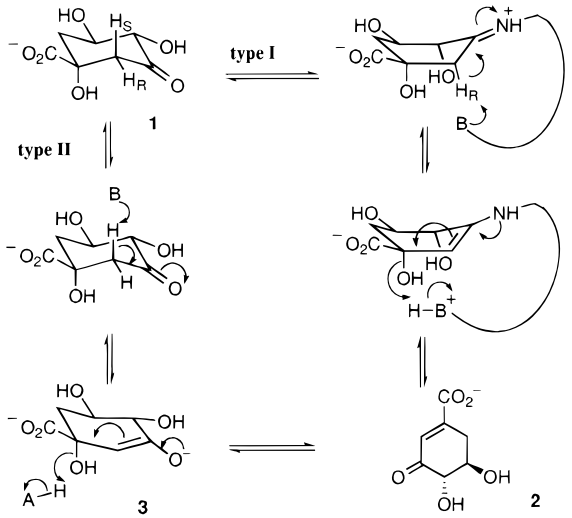
Table 1. Inhibition Constants (μM) for Inhibitors against Type I and Type II Dehydroquinases

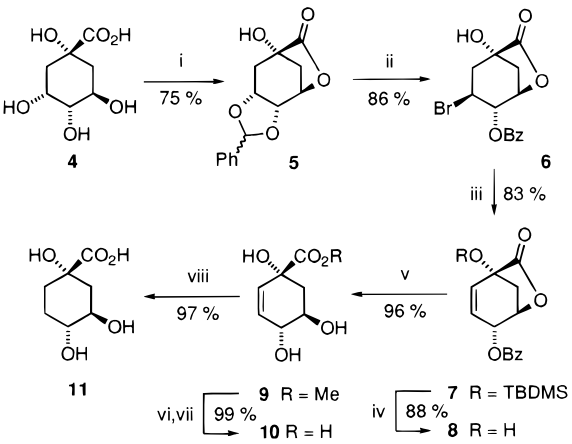
enzyme	K_m[a]	K_i for **10**	K_i for **11**	K_i for **13**	K_i for **19**
type I *S. typhimurium*	18[19]	3000 ± 1 000	4500 ± 500	>25 000	>25 000
type II *A. nidulans*	150[3]	60 ± 10	1500 ± 200	2 200 ± 100	15 ± 2
type II *M. tuberculosis*	64[7]	200 ± 20	1200 ± 200	700 ± 200	20 ± 2
type II *S. coelicolor*	650[9]	30 ± 10	600 ± 200	2 500 ± 500	500 ± 200

[a] The K_m values (μM) were measured for the respective enzymes with dehydroquinic acid **1**.

Scheme 1



Scheme 2[a]

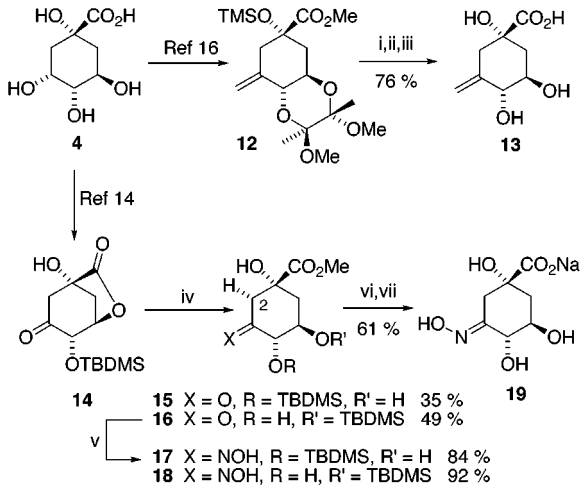


[a] Reagents and conditions: (i) PhCHO, TsOH·H_2O, PhMe, 110 °C, 16 h; (ii) NBS, AIBN, C_6H_6, 80 °C, 1 h; (iii) TBDMSCl, DBU, MeCN, 80 °C, 16 h; (iv) Bu_4F, THF, 20 °C, 30 min; (v) NaOMe, MeOH, 20 °C, 2 hg; (vi) NaOH, H_2O, 20 °C, 30 min; (vii) Amberlite IR-120 (H), H_2O, 20 °C, 10 min; (viii) H_2, Pt, H_2O, 20 °C, 16 h.

All the compounds were poor inhibitors against the type I enzyme. The *exo*-methylene compound **13** and the oxime **19** were particularly poor, presumably due to steric interactions with the C-3 substituent, possibly from the active-site lysine involved in imine formation. 2,3-Anhydroquinic acid **10** was a reasonably good inhibitor against all three type II enzymes, especially that from *Streptomyces*. Reduction of the 2,3-double bond led to a marked loss in affinity for **11.** The two inhibitors functionalized at C-3 showed contrasting inhibition against the type II enzymes. The exocyclic methylene compound **13** had approximately millimolar affinity for the type II dehydroquinases. In comparison, the oxime **19** was a particularly potent inhibitor of the *A. nidulans* and *M. tuberculosis* enzymes, but was surprisingly much less potent against the *Streptomyces* enzyme.[18]

These results broadly supported our initial assumptions. The selectivity shown by the type II enzymes between **10** and **11** for the compound with sp^2 geometry at both C-2 and C-3 is consistent with stabilization by the enzyme of a transition state that has some degree of ring flattening. Observations concerning similar geometric selectivity for the enzyme 3-dehydroquinate synthase have been reported recently.[16] Likewise, the positioning of the oxime group into the pocket on the enzyme thought to stabilize the developing negative charge in the enolate intermediate led to significantly higher affinity for **19** than for the *exo*-methylene compound **13**.

Scheme 3[a]



[a] Reagents and conditions: (i) TFA, H_2O, 60 °C, 2 h; (ii) NaOH, H_2O, 20 °C, 30 min; (iii) Amberlite IR-120 (H), H_2O, 20 °C, 10 min; (iv) NaOMe, MeOH, 20 °C, 1 h; (v) $HONH_3Cl$, NaOAc, MeCN, H_2O, 20 °C, 16 h; (vi) Bu_4NF, THF, 20 °C, 16 h; (viii) NaOH, H_2O, 20 °C, 10 min.

This is the first report of inhibitors of type II dehydroquinase. Furthermore, the inhibition constants for **19** against the *A. nidulans* enzyme and for **10** against the *S. coelicolor* enzyme are over 1 order of magnitude lower than the corresponding K_m values for the substrate dehydroquinate. This is an encouragingly high affinity for the first generation of inhibitors but is not as high as might be expected for true transition-state mimics. The results suggest that compounds combining the separate strategies of flattening the ring and having a hydrogen-bonding capability at C-3 should be interesting targets.

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Supporting Information Available: Experimental data for all new compounds and inhibition data.

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(18) Compound **19** is not a substrate for type II dehydroquinase, nor was deuterium exchange into C-2 observed when it was incubated with the type II enzyme from *S. coelicolor* or *M. tuberculosis*.

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