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Design, Synthesis, and Structural Studies on Potent Biaryl Inhibitors of Type II Dehydroquinases

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The shikimate pathway is the biosynthetic route to the aromatic amino acids phenylalanine, tryptophan, and tyrosine, and other important aromatic compounds.[1–3] Plants, fungi, bacteria, and protozoa all use this seven enzyme pathway to make chorismate. Notably, in plants over 20% of the carbon flux passes through the pathway, making it a key herbicide target (for example, Glyphosate).[1] The absence of the pathway in mammals has made the constituent enzymes attractive targets for the development of antimicrobial therapeutics.[2]

The third step of this pathway is the dehydration of 3-dehydroquinate to 3-dehydroshikimate catalysed by dehydroquinase (3-dehydroquinate dehydratase, EC 4.2.1.10). Two mechanistically and structurally distinct enzymes have evolved to catalyse this reaction, type I and type II dehydroquinase.[4] The type I dehydroquinases are dimeric proteins with a 26–28 kDa subunit that catalyse the *syn* dehydration of **1** by the initial formation of a Schiff base with a conserved lysine residue. In contrast, type II enzymes are dodecamers with smaller subunits (12–18 kDa) that catalyse the *anti* elimination of water, the reaction proceeding through an enolate intermediate (Scheme 1). The deprotonation to form the enolate is carried out by a tyrosine residue on a mobile loop, the pK_a of which is lowered by two flanking arginine residues. Type II dehydroquinases are present in several organisms including *Mycobacterium tuberculosis*[5] and *Helicobacter pylori*,[6] making this enzyme a medicinally important target.

The first reported inhibitor of type II dehydroquinase was the anhydroquinate **3** ($K_i = 30\ \mu M$, Figure 1), designed to mimic the flattened enolate intermediate (Scheme 1).[7] Significantly, it was 20-fold more potent than the corresponding reduced analogue against *Streptomyces coelicolor* type II dehydroquinase, highlighting the importance of sp^2 hybridisation between C2 and C3 for potent inhibition. Co-crystallisation of **3** with *S. coelicolor* dehydroquinase revealed a second binding pocket adjacent to the active site, occupied by a glycerol molecule (an artefact from the crystallisation process, PDB code: 1GU1, Figure 2).[8] This finding led to the search for novel inhibitors that maintained the dehydroquinate core but incorporated side chains at C3 or C4, to extend into the glycerol binding pocket.[9–14]

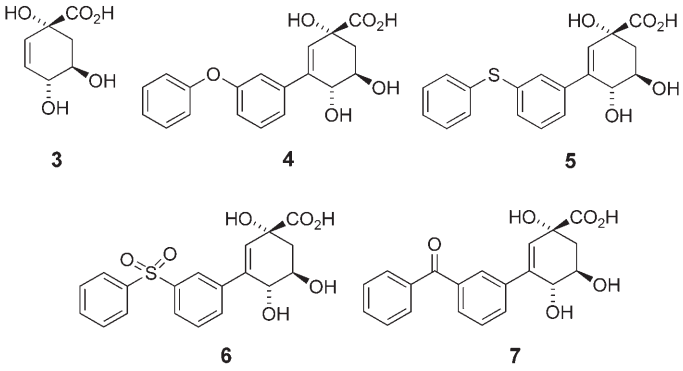
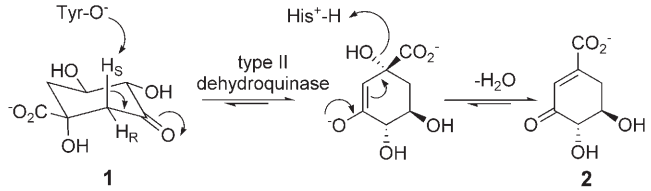


Figure 1. Anhydroquinate analogue **3** and target inhibitors **4**–**7**.

In this paper, we describe the rational design and synthesis of several novel inhibitors of type II dehydroquinase. Ana-



Scheme 1. Mechanism of type II dehydroquinases.

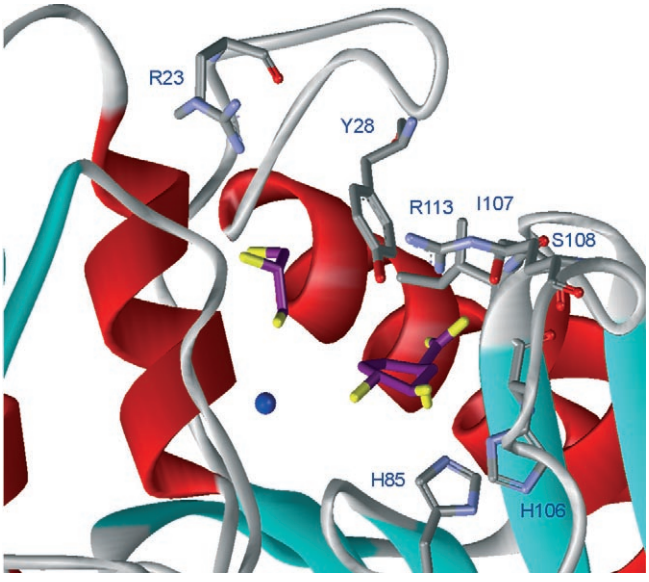


Figure 2. Crystal structure of *S. coelicolor* type II dehydroquinase with **3** and glycerol bound in the active site.[8]

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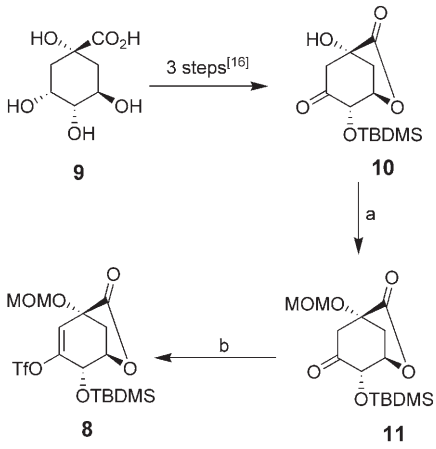
logues **4**–**7** contain the key anhydroquinate ring found in **3** with a range of biaryl side chains at C3, designed to extend into the glycerol-binding pocket and bind to amino acid side chains in this region (Figure 1). The aromatic ring directly attached to the C3 position provides a rigid link to the terminal phenyl ring. This is incorporated to interact with residues on the flap that comes down to enclose the substrate and complete the active site. Specifically this terminal phenyl ring may form a π-stacking interaction with the catalytically important tyrosine and a potential cation-π interaction with the arginine on the mobile loop. The ether, thioether, sulfone, and carbonyl functionality between the two rings on **4**–**7** afford the possibility of further interactions with the protein.

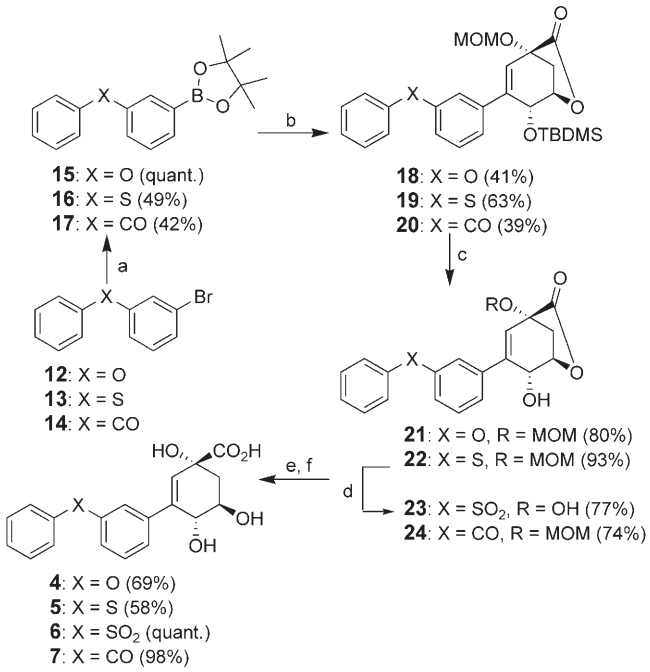
The structures **4**–**7** were docked into the active site of *S. coelicolor* type II dehydroquinase (1GU1)[8] to predict binding modes and hence, determine the suitability of the compounds as inhibitors of the enzymes. The compounds and the receptor (*S. coelicolor* type II dehydroquinase) were prepared using SYBYL7.1[15] and molecular dockings were carried out using GOLD (version 3.0).[16] All four compounds docked reproducibly with the anhydroquinate core in a similar position to that observed for X-ray structures and docking studies of previously synthesised inhibitors (see Supporting Information).[9–14]

The synthesis of target inhibitors **4**–**7** was proposed by Suzuki–Miyaura cross-coupling chemistry. The idea was to incorporate the desired biaryl substituents at C3 from the key enol-triflate intermediate **8**. Triflate **8** was prepared in two steps from the previously reported carbolactone **10** (Scheme 2).[13,17] Methoxymethyl protection of the free tertiary alcohol in **10** was achieved by treatment with dimethoxymethane and phosphorus pentoxide. Deprotonation of the resulting ketone **11** with lithium hexamethyldisilazide followed by treatment with triflic anhydride, gave the desired enol-triflate **8** in 73% yield over the two steps.

Biaryl-boronate esters were required as coupling partners in the proposed Suzuki–Miyaura reactions. Boronate-pinacolato esters **15**–**17** were synthesised from the corresponding bromides **12**–**14** under the Miyaura borylation conditions (Scheme 3).[18] Palladium-catalysed Suzuki–Miyaura cross-coupling reactions of **8** with boronate esters (**15**–**17**) were carried out in the presence of $Pd(PPh_3)_4$ and aqueous sodium carbonate to give **18**–**20** in 39–63% yields. Cleavage of the silyl-protecting groups with TBAF furnished alcohols **21**, **22**, and **24**. At this stage thioether analogue **22** was oxidised to the corresponding sulfone with hydrogen peroxide in acetic acid. This occurred with concomitant cleavage of the methoxymethyl group to give diol **23** in 77% yield. Hydrolysis of the methoxymethyl protecting groups, followed by opening of the lactone ring with aqueous sodium hydroxide, gave the desired inhibitors **4**–**7** in good yields.



Scheme 2. Synthesis of enol-triflate a) dimethoxymethane, P_2O_5, CCl_4, 98%, 22 °C; b) LiHMDS, Tf_2O, CCl_4, 0 °C, 74%; LiHMDS = lithium hexamethyldisilazide.



Scheme 3. Synthesis of biaryl inhibitors, a) Bis(pinacolato)diboron, Pd(dppf)Cl_2·CH_2Cl_2, KOAc, DMSO, 85 °C; b) enol-triflate **8**, Na_2CO_3, $Pd(PPh_3)_4$, DME, 100 °C; c) TBAF, THF, 0 °C; d) AcOH, H_2O_2, 100 °C; e) TFA, 0 °C; f) 1) NaOH, THF/H_2O, 2) amberlite IR120 (H^+); DME = dimethoxyethane, TBAF = tetrabutylammonium fluoride.

The biological activity of the inhibitors against *S. coelicolor*, *M. tuberculosis*, and *H. pylori* type II dehydroquinases was determined using a UV spectrophotometric assay measuring the initial rate of product (3-dehydroshikimate, **2**) formation by detecting the enone-carboxylate chromophore at 234 nm. The K_i values were obtained using the kinetics software GraFit[19] and the inhibition data are summarised in Table 1. All the compounds were competitive reversible inhibitors of the three enzymes as shown by least squares fitting to a competitive model (see Supporting Information).

Compounds **4** and **5**, containing diphenylether and diphenylsulfide substituents at C3 respectively were nanomolar inhibitors of *S. coelicolor* type II dehydroquinase. Analogue **4** (K_i = 10 nM) is one of the most potent inhibitors of any dehydroquinase reported. Replacement of the ether linkage with a

Table 1. Inhibition constants (K_i) of **4**–**7** against type II dehydroquinase from *S. coelicolor*, *H. pylori*, and *M. tuberculosis*.[a]

Inhibitor	*S. coelicolor* type II [nM]	*H. pylori* type II [nM]	*M. tuberculosis* type II [nM]
4	10 ± 0.2	490 ± 60	740 ± 70
5	70 ± 0.4	1200 ± 100	380 ± 30
6	2700 ± 200	24000 ± 5200	> 100000
7	4700 ± 400	2900 ± 300	11000 ± 1000

[a] *S. coelicolor* (K_m = 129 ± 20 μM, k_{cat} = 136 s^{-1}), *M. tuberculosis* (K_m = 25 ± 5 μM, k_{cat} = 3.6 s^{-1}), and *H. pylori* (K_m = 177 ± 20 μM, k_{cat} = 0.93 s^{-1}).

sulfide in **5** (K_i = 70 nM) led to a seven-fold drop in potency. Analogues **4** and **5** were also potent inhibitors of the *H. pylori* enzyme where the diphenylether analogue **4** (K_i = 490 nM) is only two-fold more potent than the corresponding diphenylsulfide analogue **5** (K_i = 1.2 μM). Analogue **4** represents the most potent inhibitor of *H. pylori* type II dehydroquinase synthesised to date.

Docking studies have shed some light on why replacement of oxygen with sulfur at the biaryl bridge position affects potency. The main chain carbonyl of Asn16 is very close to the bridging oxygen (2.80 Å) and sulfur (2.55 Å) atoms in the dockings (Supporting Information). It is presumed that the closeness of the sulfur, and its larger orbital size, result in greater repulsion by the lone pairs of the carbonyl oxygen than is experienced by the ether link in **4**. This pattern of inhibition is reversed for **4** and **5** against *M. tuberculosis* dehydroquinase. The compounds were both nanomolar inhibitors of the enzyme, however diphenylsulfide analogue **5** (K_i = 380 nM), is two-fold more potent than **4** (740 nM). The disordered nature of the flexible loop in crystal structures of the *M. tuberculosis* enzyme mean it has not been possible to study inhibitor binding in detail by inspection of docked structures. However, these variations in potency suggest subtle differences in the glycerol-binding pockets of the three type II enzymes that could be used in making organism specific inhibitors.

Analogues **6** and **7**, which have diphenylsulfone and benzophenone side chains respectively, were significantly less potent than **4** and **5** against all three type II dehydroquinases. Compound **7** exhibited low micromolar inhibition constants against all three enzymes, whereas **6** was a micromolar inhibitor of the *S. coelicolor* and *H. pylori* enzymes but showed no measurable inhibition of *M. tuberculosis* type II dehydroquinase. It is clear from the inhibition results that an increase in steric bulk at the biaryl-bridge position is detrimental to high affinity binding of these compounds to the enzymes.

To quantify the binding mode of these inhibitors, thioether analogue **5** was co-crystallised with *S. coelicolor* type II dehydroquinase and a crystal structure obtained at 2.2 Å resolution (Supporting Information). The crystal structure shows the anhydroquinate core bound in the active site and the C3 side chain extending into the glycerol-binding pocket (Figure 3).[20] The binding mode was very similar to that observed in the docking studies of this compound (RMSD = 0.46 Å), validating the use of a rational design approach in these inhibition studies.

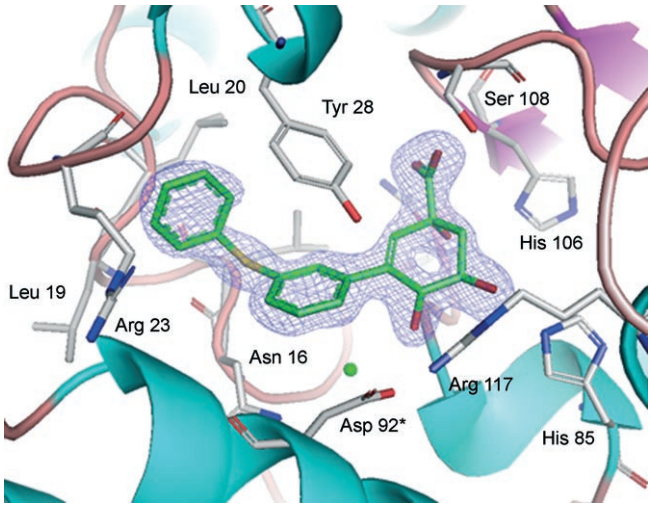


Figure 3. The crystal structure of *S. coelicolor* type II dehydroquinase with **5** occupying the active site and glycerol-binding pocket. Unaveraged electron density for the ligand is shown at the two sigma level for a representative monomer.

It is instructive to compare the crystal structure of *S. coelicolor* type II dehydroquinase in complex with **5** to the previously described structure with **3** bound.[8] Diphenylsulfide analogue **5** has the same anhydroquinate core as **3**, however, in the structure of **5** the ring is tilted around C5 in the plane of the ring over 20° so that C3 is raised 0.9 Å relative to the crystal structure of **3.** This movement is attributed to the extent and rigidity of the C3 biaryl substitution. Despite this movement of the anhydroquinate core, comparable hydrogen-bonded interactions are maintained. The phenyl ring directly attached to C3 makes favourable van de Waals contacts with Asp92* and displaces a conserved water seen in other structures.[8,11] The thioether packs against the amide bond between Asn16 and Leu17, and the terminal phenyl ring packs against the peptide bond between Leu19 and Leu20 as well as the side chains of Arg23 and Tyr28 and makes a number of short contacts to an ordered water in the lid domain and the carbonyl oxygens of Asn16 and Leu19. The guanadino group of Arg23 is not involved in a cation-π interaction with the terminal phenyl ring of **5**, in part due to it forming a salt bridge with Asp98*, an interaction seen in a large number of the *S. coelicolor* type II dehydroquinase structures. An edge-on π-stacking interaction of the terminal phenyl ring with Tyr28 of the flexible loop can be clearly observed in the crystal structure and is assumed to contribute to the enhanced potency of **5** compared to **3**.

In summary, we have reported the rational design and synthesis of four novel inhibitors of type II dehydroquinases containing a variety of biaryl side chains attached to an anhydroquinate core. All the compounds proved to be significantly more potent than the anhydroquinate **3** against *S. coelicolor* type II dehydroquinase. Compounds **4** and **5** with biaryl-ether and biaryl-thioether substituents at C3 were potent inhibitors of all three enzymes. Compound **4** was a 10 nM inhibitor of *S. coelicolor* type II dehydroquinase, one of the most potent inhibitors of any dehydroquinase synthesised to date. This compound also exhibited potent inhibition of the *H. pylori* enzyme

($K_i = 490$ nM), representing the most active compound reported against this medicinally relevant enzyme.

The binding mode of **5** in the crystal structure of *S. coelicolor* type II dehydroquinase was consistent with molecular docking studies and supported the hypothesis that an edge-on stacking interaction with Tyr 28 of the flexible loop was responsible for the increase in potency. Future studies will focus on crystallisation of these compounds with the *M. tuberculosis* and *H. pylori* type II dehydroquinases to deduce the ordered binding pockets of these enzymes. This will aid in the future design of organism specific enzyme inhibitors.

Two of the compounds reported in this study (**4** and **5**) showed significant in vivo activity in preliminary screens against *M. aurum*, a model system for *M. tuberculosis*.[21] It is hoped that these studies will give impetus to the development of new type II dehydroquinase inhibitors with the ultimate goal of developing new broad spectrum antimicrobials.

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[20] PDB code 2CJF for coordinates and structure factors R2CJFSF.
[21] In vivo data against *M. aurum* will be reported in due course.

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