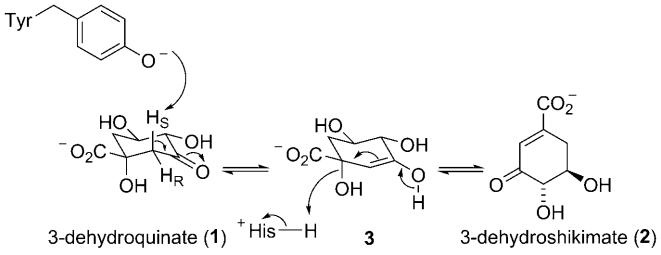
DOI: 10.1002/cmdc.201000399

Synthesis and Evaluation of Potent Ene–yne Inhibitors of Type II Dehydroquinases as Tuberculosis Drug Leads

Anh Thu Tran,[a] Katie M. Cergol,[a] Nicholas P. West,[b] Elizabeth J. Randall,[b] Warwick J. Britton,[b] Syed Ali Imran Bokhari,[c] Musadiq Ibrahim,[c] Adrian J. Lapthorn,[c] and Richard J. Payne*[a]

The shikimate pathway is an essential biosynthetic route operating in plants, bacteria, fungi, and apicomplexan parasites.[1–4] Consisting of seven enzyme-catalyzed steps, this pathway is responsible for the sequential conversion of erythrose-4-phosphate and phosphoenol pyruvate to chorismate, the precursor for the biosynthesis of a range of aromatic amino acids, folates, ubiquinone, and vitamins E and K.[1,5,6] The absence of this pathway in mammals, alongside its essential role in the survival of plants, bacteria, fungi and apicomplexan parasites, makes this an attractive target for the development of new herbicides and antimicrobial agents.[1]

The enzyme dehydroquinase (3-dehydroquinate dehydratase, EC 4.2.1.10) catalyzes the third step in the shikimate pathway—the reversible dehydration of 3-dehydroquinate (**1**) to 3-dehydroshikimate (**2**). Two structurally distinct dehydroquinase (DHQase) enzymes are known (type I and II), which catalyze the same dehydration reaction via two mechanistically distinct pathways.[7,8] Type I dehydroquinases are functional dimers that catalyze the *syn*-elimination of water via an imine intermediate with a conserved lysine residue, which enables the abstraction of the *pro*-R hydrogen from the C-2 position (H_R, Scheme 1).[9,10] In contrast, the type II dehydroquinases are dodecamers that facilitate the *anti*-elimination of water via an E_{1CB} mechanism, whereby abstraction of the more acidic *pro*-S hydrogen (H_S, Scheme 1) is facilitated by a conserved tyrosine residue, resulting in the formation of an enol intermediate **3** (Scheme 1).[7,11,12]



Scheme 1. Proposed mechanism for the dehydration of 3-dehydroquinate (**1**) to 3-dehydroshikimate (**2**) by type II dehydroquinases.

Type II dehydroquinases are present in pathogenic bacteria such as *Mycobacterium tuberculosis*,[13] the etiological agent of tuberculosis (TB), and *Helicobacter pylori*,[14] a stomach pathogen that causes gastric ulcers and is linked to the development of stomach cancer. Due to the increased resistance of these and other microorganisms to current chemotherapies, antibacterial agents with novel mechanisms of action are required. Since the 1990s, several new inhibitors of type II dehydroquinases have been reported.[15] In particular, work by González-Bello et al.[16–19] and Abell et al.[20–22] has highlighted several specific features that contribute to the potent inhibition of this enzyme. In particular, compounds possessing an anhydroquinate core, mimicking the enol intermediate **3**, attached either directly to an aromatic moiety (e.g., **4**,[18] Figure 1), or via an in-

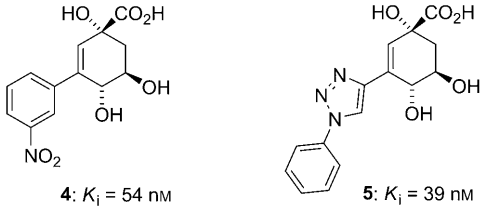


Figure 1. Potent inhibitors of *M. tuberculosis* type II dehydroquinase.[17,22]

flexible linker, have proven successful. More recently, we have reported potent inhibitors that support this design strategy, incorporating a rigid triazole linker between the anhydroquinate core and a range of aromatic groups (e.g., **5**, Figure 1).[23] This design of small molecules is supported by reported co-crystal structures of inhibitors bound to the active site of type II dehydroquinases from *H. pylori* and *Streptomyces coelicolor*.[17,20] More recently, González-Bello and co-workers reported the first co-crystal structure of *M. tuberculosis* type II dehydroquinase with a substrate-based inhibitor bound in the active site.[24] This data further highlights the role of the quinate core, which forms several hydrogen bonds with active site residues, and terminal aromatic substituents, which participate in favourable π-stacking interactions with a conserved tyrosine residue within a flexible loop.

We have become interested in the development of type II dehydroquinase inhibitors as potential tuberculosis drug leads, which would operate via a mode of action that is different to currently employed therapies. In this study, we report the development of a range of ene–yne-based inhibitors **6**–**13** cen-

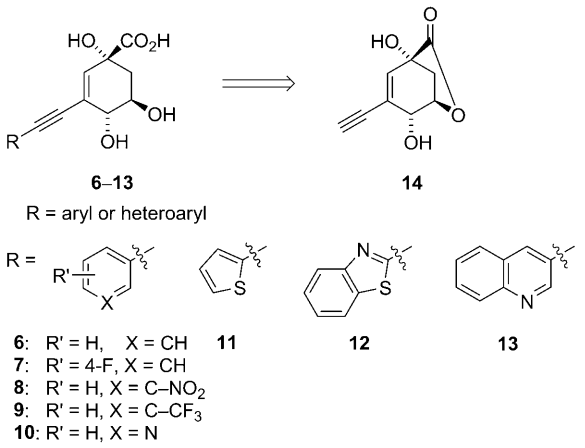
[a] *A. T. Tran, Dr. K. M. Cergol, Dr. R. J. Payne*
School of Chemistry F11, The University of Sydney, Sydney, 2006 (Australia)
Fax: (+61) 2-9351-3329
E-mail: richard.payne@sydney.edu.au

[b] *Dr. N. P. West, E. J. Randall, Prof. W. J. Britton*
Mycobacterial Research Program, Centenary Institute of Cancer Medicine and Cell Biology, NSW, AUSTRALIA and Sydney Medical School
The University of Sydney, NSW 2006 (Australia)

[c] *S. A. I. Bokhari, M. Ibrahim, Dr. A. J. Lapthorn*
Department of Chemistry and Division of Biochemistry and Life Science
University of Glasgow, G128QQ (UK)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201000399.

tred on the key inhibitory features described above (Scheme 2). We envisioned that these inhibitors could be prepared in an efficient manner by employing a palladium-catalyzed Sonogashira cross-coupling reaction[25,26] between a key



Scheme 2. Retrosynthesis of ene–yne inhibitors **6**–**13**.

ene–yne intermediate **14** and several aromatic moieties encompassing a range of electronic and steric properties (Scheme 2). In this case, the alkyne functionality serves as a rigid, linear, hydrophobic linker between the anhydroquinate core and the aromatic moieties, a strategy which has yet to be exploited for type II dehydroquinase inhibition.

To support our inhibitor design, the proposed ene–yne compounds **6**–**13** were initially docked into the active site of the type II dehydroquinases from *S. coelicolor* (PDB code: 1GU1) and *H. pylori* (PDB code: 2WKS) using Glide (Schrödinger Inc.).[27] Most inhibitors possess a common predicted binding mode whereby the anhydroquinate core formed a number of favourable hydrogen-bonding interactions with active site residues (as has been observed in previous crystal structures of anhydroquinate-containing inhibitors, see Supporting Information).[17,20] In addition, the rigid alkyne linker appeared to orient the terminal aryl and heteroaryl moieties so as to achieve a favourable π-stacking interaction with the essential tyrosine residue (Tyr28 in *S. coelicolor* and Tyr22 in *H. pylori*). Representative docking poses of **9**, bearing a terminal *m*-trifluoromethylphenyl substituent, depicting this common binding mode are shown in Figure 2. Compounds **12** and **13**, containing terminal benzothiazole and quinoline groups, appeared to dock in a modified mode against the *S. coelicolor* enzyme whereby the anhydroquinate core was oriented 180 degrees from the normal pose (see Supporting Information). It is assumed that this is due to the steric nature of the terminal bicyclic rings, which, in order to partake in a favourable π-stacking interaction with the conserved tyrosine residue, must present the anhydroquinate core in a different orientation.

Having established that the proposed inhibitors provided favourable binding modes in silico, we next undertook the synthesis of key ene–yne **14**, and the subsequent elaboration to inhibitors **6**–**13**. To this end, ene–yne **14** was first prepared

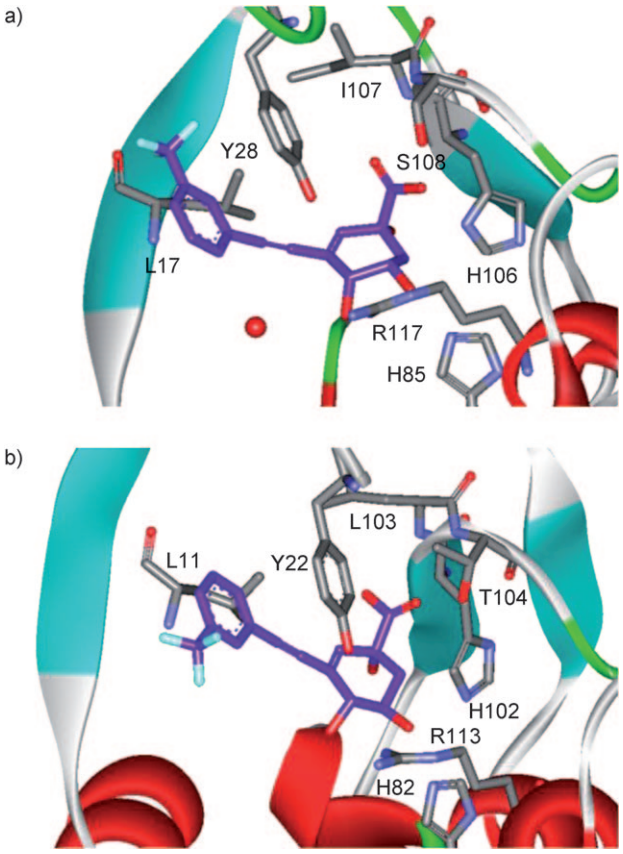
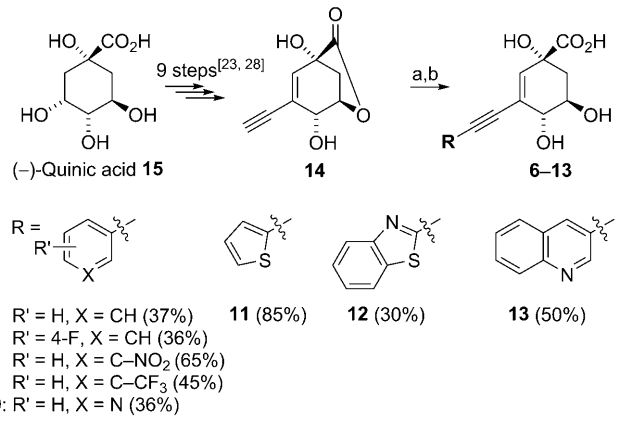


Figure 2. Molecular docking of **9** into the active site of a) *S. coelicolor* type II dehydroquinase (PDB code: 1GU1) and b) *H. pylori* type II dehydroquinase (PDB code: 2WKS).

from commercially available (−)-quinic acid (**15**) in nine steps (Scheme 3).[23,28] With the key ene–yne **14** in hand, we directed our attention towards the synthesis of the proposed ene–yne inhibitors **6**–**13** (Scheme 3). Subjecting ene–yne **14** to palladium-catalyzed Sonogashira cross-coupling reaction conditions with a variety of aryl halides or triflates afforded the precursor lactones that, upon saponification, resulted in the desired in-



Scheme 3. Synthesis of ene–yne inhibitors **6**–**13** from (−)-quinic acid. *Reagents and Conditions*: a) R-X (X = Br, I, or OTf), $Pd(PPh_3)_4$, CuI, piperidine, THF, 40 °C; b) LiOH, THF/H_2O, RT, 30–85% (two steps).

hibitors **6**–**13** in moderate to good yields over the two steps (30–85 %).

Compounds **6**–**13** were next screened against type II dehydroquinases from three organisms, namely *S. coelicolor*, *H. pylori* and *M. tuberculosis*. The inhibitors were tested using a UV spectrophotometric assay, detecting the enone carboxylate chromophore of the enzymatic product 3-dehydroshikimate (**2**) at $\lambda = 234$ nm.[21] Gratifyingly, the majority of the compounds prepared in this study proved to be potent inhibitors of all three of the type II dehydroquinases studied, with inhibition constants in the nanomolar range. Introduction of terminal phenyl or 4-fluorophenyl substituents, in **6** and **7** respectively, led to potent inhibition of all three of the type II dehydroquinases (Table 1). Both compounds were low nanomolar inhibitors of the *S. coelicolor* enzyme ($K_i = 12$–28 nM); however, they were less potent against the *M. tuberculosis* enzyme with inhibition constants of 356 nM and 548 nM for **6** and **7**, respectively. This phenomenon has been reported for previously prepared anhydroquinate-based inhibitors, suggesting subtle differences in the active site structures of these two enzymes.[19,20,22] Compounds **8** and **9** bearing electron withdrawing *m*-trifluoromethylphenyl and *m*-nitrophenyl substituents, respectively, exhibited the most potent inhibition of all three of the enzymes. Notably, compounds **8** and **9** exhibited inhibition constants of 133 nM and 108 nM, respectively, against *M. tuberculosis* type II dehydroquinase and, as such, represent two of the most potent inhibitors ever tested against this enzyme. It is important to note that compounds **8** and **9** were also potent inhibitors of *H. pylori* type II dehydroquinase with inhibition constants of 98 nM and 103 nM, respectively. To our knowledge, these are the most potent inhibitors ever reported against this enzyme. Introduction of a 3-pyridyl and 3-quinoline moiety in **10** and **13**, respectively, led to a significant drop in potency against all three enzymes, suggesting that introduction of nitrogen heterocycles is detrimental to inhibition of the type II dehydroquinases. Interestingly, compounds **11** and **12** bearing terminal thiophene and benzothiazole rings also led to potent inhibition of all three enzymes including *M. tuberculosis* type II dehydroquinase.

Table 1. Inhibition constants of ene–yne inhibitors **6**–**13** against *S. coelicolor*, *H. pylori*, and *M. tuberculosis* type II dehydroquinase enzymes.[a]

Inhibitor	Type II DHQase K_i [nM]		
	S. coelicolor	*H. pylori*	*M. tuberculosis*
6	28 ± 3	253 ± 33	356 ± 23
7	12 ± 1	106 ± 18	548 ± 64
8	2.2 ± 0.4	98 ± 16	133 ± 15
9	6.9 ± 0.9	103 ± 13	108 ± 10
10	171 ± 23	712 ± 137	2194 ± 404
11	7.1 ± 0.8	316 ± 44	187 ± 18
12	7.5 ± 0.7	94 ± 11	126 ± 9
13	168 ± 20	1203 ± 134	885 ± 67

[a] Kinetic constants: *S. coelicolor*: $K_M = 210 \pm 32$ μM, $k_{cat} = 61$ s^{-1}, 50 mM Tris·HCl, pH 7.0, 25 °C; *H. pylori*: $K_M = 75 \pm 10$ μM, $k_{cat} = 0.34$ s^{-1}, 50 mM Tris·HOAc, pH 7.0, 25 °C; *M. tuberculosis*: $K_M = 36 \pm 4$ μM, $k_{cat} = 4.6$ s^{-1}, 50 mM Tris·HCl, pH 7.0, 25 °C.

Having prepared a library of type II dehydroquinase inhibitors with nanomolar affinity, we were interested in assessing the antibacterial activity of these compounds. Specifically, we chose to test the activity of **6**–**13** against virulent *M. tuberculosis* (strain H37Rv) to assess the potential of these compounds as TB drug leads. The activity against replicating *M. tuberculosis* was measured using the well-established microplate-based assay with Alamar blue (resazurin) readout (MABA) for determination of bacterial growth (see Supporting Information).[29–32] Although the inhibitors demonstrated activity, the majority of the compounds exhibited low millimolar IC_{50} values against the growth of *M. tuberculosis*, significantly less potent than the in vitro activity against type II dehydroquinase from *M. tuberculosis* (see Supporting Information). The most potent inhibitor of *M. tuberculosis* growth was compound **8** bearing an *m*-nitrophenyl moiety, which exhibited an IC_{50} value of 450 μM and a minimal inhibition concentration (MIC) of 1.2 mM (see Supporting Information). A possible explanation for the reduced activity of these compounds against *M. tuberculosis* may be due to the type II dehydroquinase in the shikimate pathway of *M. tuberculosis* being non-rate limiting. As such, high levels of enzyme inhibition would be required to show significant inhibition of bacterial growth. An alternative rationale for the poor activity of these compounds is correlated with their hydrophilic nature, which may make it difficult for the compounds to traverse the waxy mycobacterial cell wall. Future work in our laboratories will therefore focus on the preparation of prodrug analogues of **6**–**13**. It is anticipated that this will enable greater concentrations of the inhibitors to enter the *M. tuberculosis* cell for inhibition of type II dehydroquinase.

In summary, we have successfully designed and synthesized a small library of ene–yne inhibitors guided by in silico docking studies. The compounds were synthesized in an efficient manner using a Sonogashira cross-coupling reaction between an alkyne-derivatized anhydroquinate core and a range of aromatic and heteroaromatic coupling partners. The vast majority of the inhibitors prepared in this study exhibited nanomolar inhibition of type II dehydroquinases from *S. coelicolor* and the medically relevant *H. pylori* and *M. tuberculosis*. The most potent inhibitors were those possessing *meta*-substituted terminal phenyl moieties bearing electron-withdrawing nitro- or trifluoromethyl-substituents. The compounds proved to exhibit only moderate antibacterial activity when screened against *M. tuberculosis*, ascribed to the hydrophilic nature of the inhibitors. Future work in our laboratories will involve increasing the lipophilicity of **6**–**13** by utilising a prodrug approach and the design of a second generation series of type II dehydroquinase inhibitors with improved cell penetrating properties.

Acknowledgements

We thank the Australian National Health and Medical Research Council for Funding (632769). We would also like to acknowledge

Ph.D. funding from an Australian Postgraduate Award and a John Lamberton Research Scholarship (ATT).

Keywords: antibiotics · dehydroquinases · medicinal chemistry · *Mycobacterium tuberculosis* · shikimate pathway

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Received: September 19, 2010
Published online on November 4, 2010

