DOI: 10.1002/cmdc.201100606

# Elucidation of *Mycobacterium tuberculosis* Type II Dehydroquinase Inhibitors using a Fragment Elaboration Strategy

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A library of novel *Mycobacterium tuberculosis* type II dehydroquinase (DHQase) inhibitors were discovered through the use of a fragment elaboration approach. Putative active site binding fragments were initially assessed in silico which led to the selection of two small aromatic fragments for further investigation. Synthetic elaboration of the fragments provided a library of 34 inhibitors that exhibited low-micromolar inhibition of type II DHQase. A number of these inhibitors also showed antibacterial activity in the low-micromolar range in screens against *M. tuberculosis* in vitro; these now serve as lead compounds for further development of therapeutics for the treatment of tuberculosis.

## Introduction

Since its initial identification in 1882, *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), has remained a major global health risk.[1] According to the World Health Organization, there were 9.4 million new cases of TB in 2009 and 1.7 million deaths, with these figures expected to rise considerably over the next decade.[1] The current situation has been attributed to poor public health management, particularly in developing countries, where there is a high TB burden and rapid spread of TB infection.[1] A further challenge for TB treatment is the ability of *M. tuberculosis* to remain dormant in macrophages in very low numbers for decades after initial infection, resulting in a lifelong risk of disease reactivation, especially in immune-compromised individuals such as those with human immunodeficiency virus (HIV) co-infection.[1,2]

In recent years, the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* has threatened global control of TB. MDR *M. tuberculosis* strains are defined as being resistant to the major first-line antitubercular agents isoniazid and rifampicin and, as such, require the use of second-line treatments such as fluoroquinolones and injectable agents (e.g., aminoglycosides and polypeptides).[3] The use of second-line drugs inevitably increases the exposure of *M. tuberculosis* to a wider range of antibacterials which has led to the emergence of XDR *M. tuberculosis* strains, defined as those resistant to isoniazid, rifampicin, and any fluoroquinolone and one of the three injectable drugs.[4] The treatment of individuals infected with XDR *M. tuberculosis* is confined to a small number of highly toxic and less effective antibiotics; as a result, XDR-TB is often untreatable, highlighted by a 98% mortality rate in a recent outbreak in South Africa.[5]

Given the alarming rise in the number of MDR and XDR *M. tuberculosis* infections combined with the high rate of HIV co-infection, there is an urgent need for new TB drugs that operate via novel modes of action to combat dwindling treatment options.[6] Among some of the promising drug targets identified by the complete analysis of the *M. tuberculosis* genome are enzymes in the shikimate pathway.[7] This biosynthetic pathway operates in plants, bacteria, fungi and apicomplexan parasites, but is absent in mammals and is therefore an attractive target for the development of small-molecule inhibitors to serve as herbicides, antibacterials, fungicides, and antiparasitic agents.[8] The shikimate pathway consists of seven enzyme-catalyzed steps and is responsible for the conversion of erythrose-4-phosphate **1** and phosphoenol pyruvate **2** into chorismate **3** and is essential for *M. tuberculosis* growth in vitro (Scheme 1).[9] Chorismate serves as a precursor for the biosynthesis of a range of aromatic amino acids, folate, ubiquinone, and vitamins E and K.[10] The presence of the shikimate pathway in *M. tuberculosis* has led to significant interest in the development of inhibitors of enzymes in this pathway to serve as TB drug leads.[11]

The third step of the shikimate pathway, the reversible dehydration of 3-dehydroquinate **4** to 3-dehydroshikimate **5**, is catalyzed by the enzyme dehydroquinase (DHQase, 3-dehydroquinate dehydratase, EC 4.2.1.10; Scheme 1). Interestingly, two structurally and mechanistically distinct enzymes (type I and type II DHQases) have evolved to catalyze the same dehydration reaction.[12] Type I DHQases are homodimeric proteins that catalyze the *syn* elimination of water via formation of a Schiff base with a conserved lysine residue, which enables abstraction of the pro-*R* hydrogen atom from the C2 position.[13] In contrast, type II enzymes are homododecamers that catalyze

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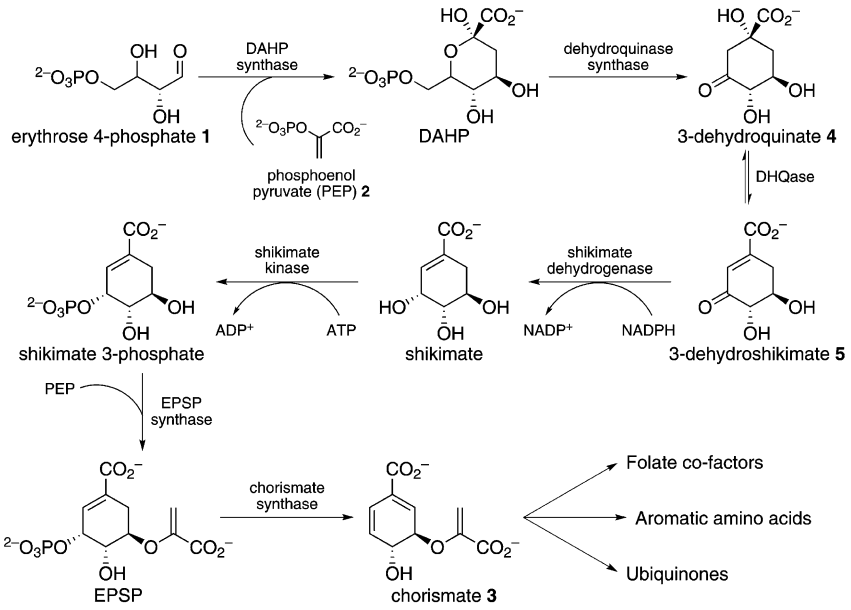
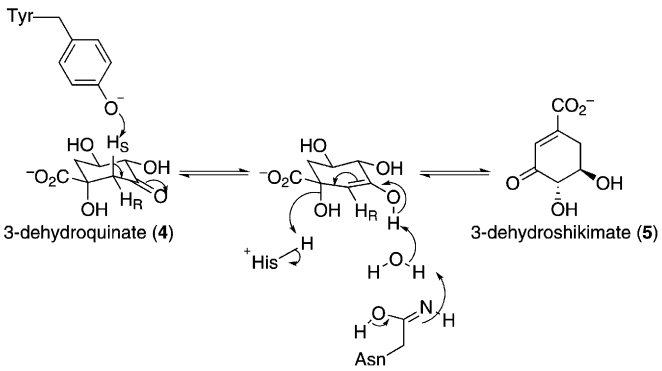
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201100606.




**Scheme 1.** The shikimate pathway.

the dehydration reaction via an $E_{1CB}$ mechanism.[14] The reaction is initiated by abstraction of the more acidic pro-*S* hydrogen atom by a conserved tyrosine residue, resulting in the formation of an enol intermediate (Scheme 2). *Anti* elimination of water then occurs, facilitated by a conserved water molecule and asparagine and histidine residues (Scheme 2).[15]




**Scheme 2.** Proposed mechanism for the dehydration of 3-dehydroquinate (**4**) to 3-dehydroshikimate (**5**) by type II DHQase.

The essentiality of type II DHQase in *M. tuberculosis* and other pathogenic bacteria such as *Helicobacter pylori* (the etiological agent of gastric ulcers) has fueled research efforts into the design and synthesis of type II DHQase inhibitors as antibacterial leads. Since the 1990s, several inhibitors of this enzyme have been reported based on the substrate and enzyme-bound intermediate. In particular, work by Abell and co-workers,[16] González-Bello and colleagues,[17] as well as from our research group[18] has identified several key features that contribute to the potent inhibition of type II DHQases from a variety of organisms.

In particular, compounds that possess an anhydroquinate core as a mimic of the enol enzyme intermediate of type II DHQase, either attached directly or by a rigid linker to a terminal aromatic moiety, have provided compounds with inhibition constants in the nanomolar range against a range of type II DHQases, including the *M. tuberculosis* enzyme (compounds **6**–**9**, Figure 1). The binding modes of these inhibitors have been predicted by molecular modeling and confirmed by enzyme–inhibitor co-crystal structures.[16c,19] These studies have established the importance of the anhydroquinate core, which forms a hydrogen bonding network with active site residues, and the terminal aromatic moieties, which participate in a π–π stacking interaction with the essential Tyr residue present on a flexible loop (Figure 2).

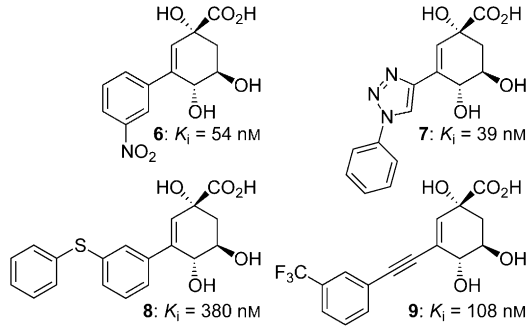



**Figure 1.** Previously synthesized inhibitors and inhibition constants against *M. tuberculosis* type II DHQase.[16c,17a,18]

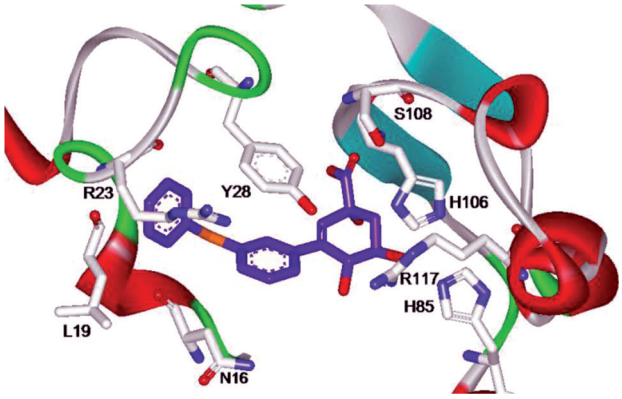



**Figure 2.** Crystal structure of *S. coelicolor* type II DHQase (PDB code: 2CJF) with thioether **8** bound.[16c]

## Results and Discussion

We recently reported a number of potent type II DHQase inhibitors that support the aforementioned design strategy through the incorporation of a rigid triazole or alkyne linker between the anhydroquinate core and a range of aromatic moieties (e.g., **7** and **9**, Figure 1).[18] Despite potent inhibition (in the nanomolar range) against *M. tuberculosis* type II DHQase, these compounds exhibited poor antibacterial activity when tested against the virulent H37Rv strain of *M. tuberculosis* in vitro.[18b] The poor antibacterial activity was attributed to the hydrophilic nature of the triol carboxylate motif present in the anhydroquinate core of these compounds. Indeed, improved in vitro antibacterial activity of anhydroquinate-based inhibitors has recently been observed by the use of a prodrug strategy, whereby the carboxylic acid of a similar class of inhibitors was derivatized as the corresponding *n*-propyl esters.[17c] Continuing our efforts to develop type II DHQase inhibitors as TB drug leads, in this study we sought to identify alternate, simplified scaffolds to replace the highly polar anhydroquinate core traditionally employed in type II DHQase inhibition studies.

### In silico fragment selection

We used an in silico screening approach to identify small, functionally simple molecules capable of establishing favorable interactions with the active site of *M. tuberculosis* type II DHQase. Specifically, we selected a total of 13 putative active site binding fragments that were present as part of a 42-compound library of proposed type II DHQase inhibitors recently reported by Kumar et al. in a virtual screening campaign (see Supporting Information).[20] The 13 aryl-based fragments with Clog*P* values ranging from −0.3 to 2.4 were docked into the active site of *M. tuberculosis* type II DHQase using Glide (Schrödinger Inc.)[21] to evaluate the suitability of these compounds as more hydrophobic replacements of the anhydroquinate core (see Supporting Information). Two fragments, namely 3,4-dihydroxyacetophenone **11** and the corresponding acetonide analogue **12**, were subsequently selected for synthetic elaboration. These two fragments were chosen based on favorable Clog*P* values and Glide XP scores, and because these scaffolds are amenable to synthetic elaboration to generate inhibitors.

The results of molecular docking studies of the two fragments are shown in Figure 3 along with the predicted binding mode of 2,3-dehydroquinate **10**. The carbonyl moiety of 3,4-dihydroxyacetophenone **11** was predicted to make hydrogen bonding interactions with the amide backbone between His 101 and Ile 102 as well as the side chain of Asn 75 in a similar manner to the C1 carboxylate and C1 hydroxy group of 2,3-anhydroquinate **10**. Importantly, 2,3-anhydroquinic acid is predicted to form other hydrogen bonding interactions that are absent in the molecular docking of **11**. These include hydrogen bonding interactions between the C1 carboxylate of **10** with the amide backbone between Ile 102 and Ser 103 and the hydroxy side chain of Ser103. Furthermore, the C1 hydroxy group of **10** was predicted to form a hydrogen bond with the side chain of His 101. Acetonide-derived acetophenone **12** was predicted to bind to *M. tuberculosis* type II DHQase in exclusively one binding mode, different from that predicted for **11**. Specifically, only the carbonyl group of the acetophenone moiety was predicted to form a hydrogen bonding interaction with the amide backbone between His 101 and Ile 102, whereas the acetonide moiety is oriented into a subsidiary binding pocket. Extra binding affinity in this pose can be attributed to a π-stacking interaction with Tyr 24. We chose to pursue inhibitors based on **12** irrespective of this altered binding mode, as incorporation of aryl groups onto the acetophenone moiety of **12** was predicted to reorient the acetonide core and provide a similar binding pose to **11** (see below). In addition, inhibitors based on this acetonide core would be synthetic intermediates en route to inhibitors based on **11**.

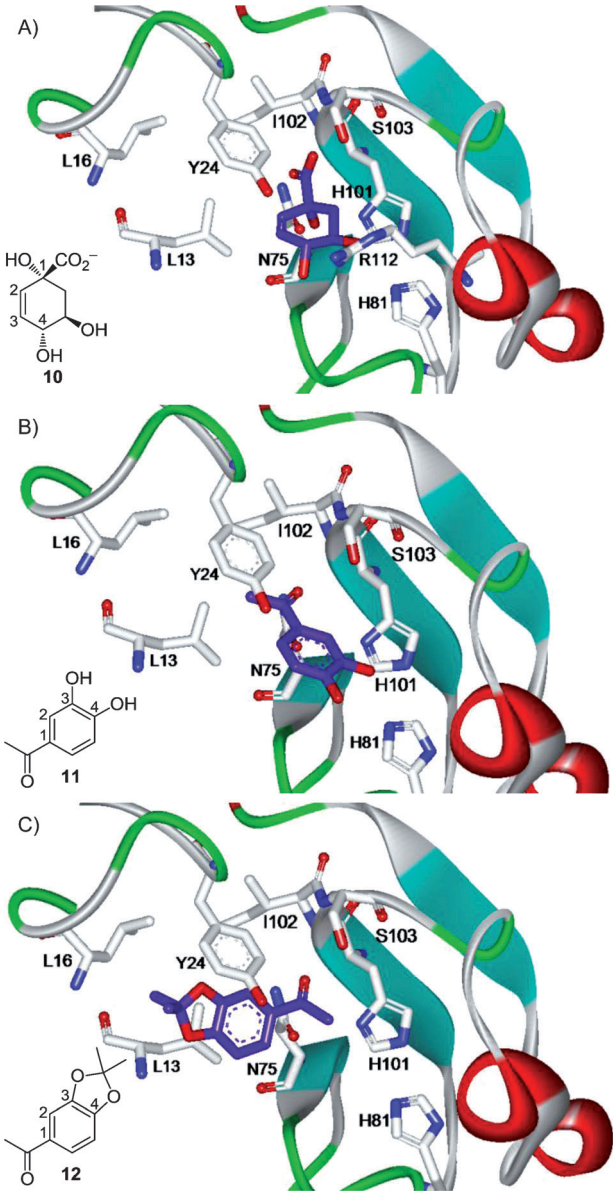
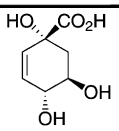
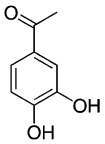
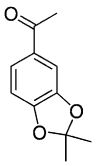



**Figure 3.** Molecular docking of A) 2,3-dehydroquinate **10**, B) 3,4-dihydroxyacetophenone **11**, and C) acetonide analogue **12** into the active site of *M. tuberculosis* type II DHQase (PDB code: 2XB8).[19a]

The suitability of these two fragments as mimics of the anhydroquinate core and as inhibitors of type II DHQase was further verified by screening for their inhibitory activity against *M. tuberculosis* type II DHQase using a continuous kinetic UV spectrophotometric assay[18a] that detects the formation of the enone–carboxylate chromophore of 3-dehydroshikimate **5** at $\lambda = 234$ nm (Table 1). We were pleased to find that compounds **11** and **12** exhibited micromolar inhibition against the enzyme

**Table 1.** Clog *P* values and activity of compounds **10**–**12** against *M. tuberculosis* type II DHQase ($K_i$) and *M. tuberculosis* strain H37Ra ($MIC_{50}$).

| | Compound | $K_i$ [μM] | $MIC_{50}$ [μM][c] | Clog *P* |
|---|---|---|---|---|
| **10** |  | 200 ± 20[a] | > 1000 | −2.7 |
| **11** |  | 173 ± 34[b] | 275 ± 49 | 1.0 |
| **12** |  | 120 ± 23[b] | 330 ± 14 | 2.4 |

[a] Inhibition constant for **10** was determined by Frederickson et al.[22] Kinetic constants for this study: $K_M = 64 \pm 6$ μM; 50 mM Tris·HCl, pH 7.0, 25 °C. [b] Kinetic constants: $K_M = 25 \pm 3$ μM, $k_{cat} = 4.5\ s^{-1}$; 50 mM Tris·HCl, pH 7.0, 25 °C. [c] Isoniazid $MIC_{50} = 176 \pm 14$ nM; rifampicin $MIC_{50} = 1.7 \pm 0.4$ nM; Glide XP scores: **10** −10, **11** −7.7, **12** −7.2. Data represent the mean ± SD from two replicate assays for *M. tuberculosis* type II DHQase and three replicate assays for *M. tuberculosis* H37Ra.

($K_i$: 173 and 120 μM, respectively). Importantly, these small molecules showed inhibition constants similar to that of 2,3-dehydroquinate **10** ($K_i = 200$ μM),[22] yet possessed significantly more favorable Clog *P* values (Table 1). Both fragments were also tested against replicating *M. tuberculosis* (H37Ra strain) using an Alamar blue (resazurin) bacterial growth assay.[23] Both **11** and **12** exhibited significant antibacterial activity ($MIC_{50}$: 275 and 330 μM, respectively) and, as such, were further confirmed as suitable fragments for chemical elaboration to generate a novel library of type II DHQase inhibitors with antitubercular activity.

### Fragment elaboration

Having demonstrated the suitability of **11** and **12** as small scaffolds with considerable inhibitory activity against *M. tuberculosis* type II DHQase, we next focused on elaborating these fragments to provide more potent inhibitors. Specifically, we chose to investigate conjugates of **11** and **12** in which a range of aryl and heteroaryl moieties were appended. These groups were designed to occupy the subsidiary binding pocket to form a favorable π–π stacking interaction with the conserved tyrosine residue (Tyr 24) present on the flexible loop, which was predicted to enhance potency. Ether and thioether linkages to **11** and **12** were proposed by implementing a broad range of aryl and heteroaryl alcohols and thiols to provide a series of inhibitors with variation in the steric and electronic nature of the terminal aryl and heteroaryl moieties (e.g., general structures **13** and **14**, Figure 4).

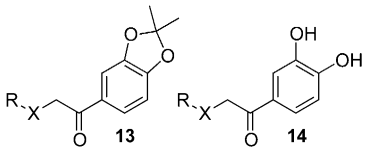


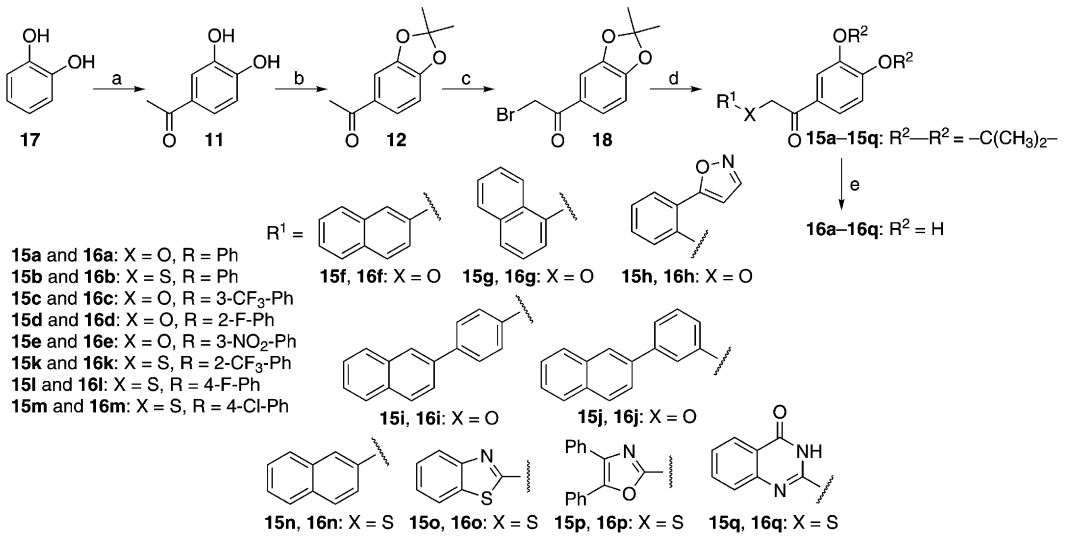

**Figure 4.** Generalized inhibitor structures bearing an acetonide (compound **13**) or catechol (compound **14**) core linked to aryl or heteroaryl rings. X = O or S; R = variety of aryl and heteroaryl groups.

A number of proposed inhibitors were assessed for suitability by the use of in silico molecular docking into the active site of *M. tuberculosis* type II DHQase studies using Glide (Supporting Information). These docking studies suggested that the majority of the inhibitors are capable of binding with the catechol and aryl acetonide moieties that occupy the active site of the enzyme, thus enabling the formation of hydrogen bonding interactions with key active site residues. Notably, the elaborated inhibitors of the aryl acetonide core (see general structure **13**, Figure 4) led to a reorientation in binding mode relative to that predicted for fragment **12** alone, such that the pose was similar to that observed for **11** and the elaborated inhibitors of this fragment. The docking studies also suggested that the terminal aromatic moieties of all inhibitors are capable of occupying the subsidiary binding site to interact with Tyr 24 (located on the flexible loop) via an offset face-to-face or edge-to-face π-stacking interaction (see the Supporting Information for binding poses).

### Synthesis

Having designed a library of target inhibitors in silico, we next undertook the synthesis of the proposed O- and S-linked inhibitors **15a**–**q** (containing the acetonide core of **12**) from catechol **17** (Scheme 3). Friedel–Crafts acylation using acetyl chloride in the presence of excess aluminum chloride provided 3,4-dihydroxyacetophenone **11** in 95% yield. Subsequent protection of the diol by treatment with acetone in the presence of excess phosphorus pentoxide furnished the acetonide-protected 3,4-dihydroxyacetophenone **12** in 40% yield. From here, bromination of **12** using copper(II) bromide in ethyl acetate and dichloromethane at reflux afforded the acetonide-protected α-bromoketone **18** in 57% yield which served as a key intermediate for the preparation of the proposed inhibitor library. Reaction of **18** with a range of aryl- and heteroaryl-derived alcohols and thiols facilitated nucleophilic substitution of the bromide and provided the 17 acetonide-based O- and S-linked inhibitors **15a**–**q** in moderate to excellent yields (59–91%). These compounds were subsequently deprotected using




**Scheme 3.** Synthesis of O- and S-linked inhibitors **15a–q** and **16a–q**. *Reagents and conditions:* a) 1. AcCl, DCE, $AlCl_3$, 10 °C→RT, 2. $HCl_{(aq)}$, 10 °C, 95%; b) $(CH_3)_2CO$, $P_2O_5$, $PhCH_3$, 75 °C, 40%; c) $CuBr_2$, EtOAc/$CH_2Cl_2$ (1:1 *v/v*), reflux, 57%; d) Ar-OH or Ar-SH, $K_2CO_3$, acetone, reflux, 4–5 h, 59–91%; e) 90% TFA, RT, 1.5 h, 57–100%.

90% aqueous trifluoroacetic acid to afford 17 catechol-based O- and S-linked inhibitors **16a–q** (Scheme 3).

### Enzyme inhibition assays

Having successfully prepared a library of 34 inhibitors, we next evaluated these compounds for their enzyme inhibitory activity. To this end, compounds **15a–q** and **16a–q** were screened against *M. tuberculosis* type II DHQase using a previously described kinetic assay.[18a] The majority of the catechol-based inhibitors **16a–q** possessed considerably enhanced inhibitory activities ($K_i$: 5–39 μM) against *M. tuberculosis* type II DHQase over compounds **11** and **12** alone ($K_i$: 173 and 120 μM, respectively; Table 2). Although the majority of the compounds clearly fitted a competitive, reversible model of inhibition, compounds **16g**, **16i**, **16j**, and **16p** fitted a mixed inhibition model tending toward a competitive mode of inhibition (alpha = 1.3–2.2, see Experimental Section and Supporting Information). This could be explained by the presence of a bulky terminal aromatic moiety in each of these compounds, which may bias interactions with the subsidiary binding pocket and enable substrate binding to the active site. Inhibitors **15a–q**, containing the acetonide core, exhibited similar inhibitory activity ($K_i$: 12–48 μM), albeit slightly less potent than **16a–q** against *M. tuberculosis* type II DHQase (Table 3). In a similar manner to the catechol-based inhibitors, the incorporation of bulky aromatic moieties generally resulted in better fitting to a mixed model of enzyme inhibition (see Experimental Section and Supporting Information). Notably, whereas fragments **11** and **12** are capable of inhibiting type II DHQase with effectiveness similar to that of 2,3-anhydroquinate **10**, the incorporation of aryl and heteroaryl groups into the acetophenone moiety resulted in—on average—a tenfold increase in inhibitory potency compared with an increase of three orders of magnitude for similar aromatic moieties appended to C3 of the anhydroquinate core in prior inhibitor studies.[16c,18a] Inspection of docking results of the inhibitors and comparison with the fragments suggests weaker hydrogen bonding interactions between the carbonyl moiety of the acetophenone core and the amide backbone between Ile102 and Ser103 in the inhibitors as a result of the newly formed π-stacking interaction. In contrast, the hydrogen bonding interactions in the active site of the anhydroquinate-based inhibitors are not reorganized if a terminal aromatic moiety is appended to facilitate a π-stacking interaction, owing to the pre-organized nature of the hydrogen bonding functionalities. This may reflect a limitation of using planar scaffolds in place of the chiral anhydroquinate core for potent inhibition of type II dehydroquinases.

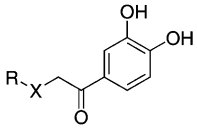
For both series of compounds (**15a–q** and **16a–q**) altering the electronic or steric properties of the aromatic moieties did not result in dramatic differences in the inhibition of the enzyme (see Tables 2 and 3). The flat structure–activity data from this study suggest that the subsidiary binding pocket of the *M. tuberculosis* enzyme is capable of accommodating a diverse range of aryl and heteroaryl rings for favorable π-stacking interactions.[16c,18a] While large variations in inhibition activity were not observed between the compounds, inhibitors **16f**, **16g**, and **16m**, bearing 2-naphthyl, 1-naphthyl, and 4-chlorophenyl moieties, respectively, exhibited the most potent inhibition of type II DHQase, with respective inhibition constants of 5, 8, and 6 μM.

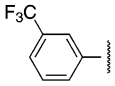
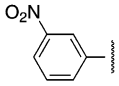
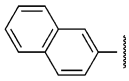
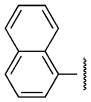
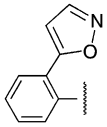
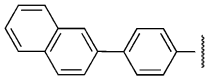
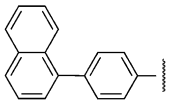
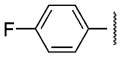
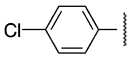
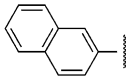
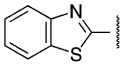
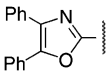
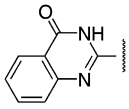
### Inhibition of *Mycobacterium tuberculosis*

Having demonstrated that the fragment-derived inhibitors are capable of exhibiting low-micromolar inhibition of *M. tuberculosis* type II DHQase in vitro, we were next interested in evaluating the in vitro activity against *M. tuberculosis* to evaluate the potential of these compounds to serve as new TB drug leads. We were pleased to observe that most inhibitors exhibited sig-

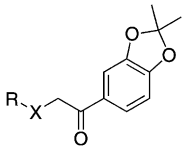
**Table 2.** Activity of acetophenone-based inhibitors **16a–q** against *M. tuberculosis* type II DHQase ($K_i$) and *M. tuberculosis* strain H37Ra ($MIC_{50}$).

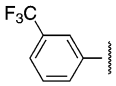
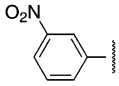
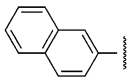
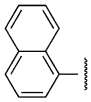
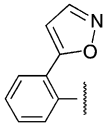
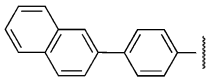
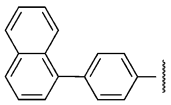
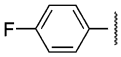
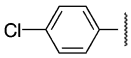
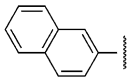
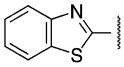
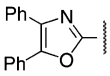
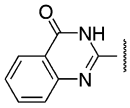


| Compd | R | X | $K_i$ [μM][a] | $MIC_{50}$ [μM][b] |
|---|---|---|---|---|
| **16a** |  | O | 18±2 | 330±14 |
| **16b** |  | S | 12±2 | 370±84 |
| **16c** |  | O | 20±3 | 505±134 |
| **16d** |  | O | 14±2 | 375±35 |
| **16e** |  | O | 13±3 | 360±14 |
| **16f** |  | O | 5±1 | 370±14 |
| **16g** |  | O | 8±2[c] | 455±7 |
| **16h** |  | O | 12±2 | 570±42 |
| **16i** |  | O | 39±9[c] | 195±49 |
| **16j** |  | O | 8±3[c] | 350±14 |
| **16k** |  | S | 36±5 | >1000 |
| **16l** |  | S | 14±3 | 485±134 |
| **16m** |  | S | 6±1 | 295±106 |
| **16n** |  | S | 14±2 | 270±1 |
| **16o** |  | S | 17±2 | >1000 |
| **16p** |  | S | 10±2[c] | 350±28 |
| **16q** |  | S | 13±2 | 690±70 |

[a] Kinetic constants: $K_M = 25 \pm 3$ μM, $k_{cat} = 4.5\ s^{-1}$; 50 mM Tris·HCl, pH 7.0, 25 °C. [b] Isoniazid $MIC_{50} = 176 \pm 14$ nM; rifampicin $MIC_{50} = 1.7 \pm 0.4$ nM. [c] Mixed model inhibition. Data represent the mean ± SD from two replicate assays for *M. tuberculosis* type II DHQase and three replicate assays for *M. tuberculosis* H37Ra.

**Table 3.** Activity of acetonide-based inhibitors **15a–q** against *M. tuberculosis* type II DHQase ($K_i$) and *M. tuberculosis* strain H37Ra ($MIC_{50}$).



| Compd | R | X | $K_i$ [μM][a] | $MIC_{50}$ [μM][b] |
|---|---|---|---|---|
| **15a** |  | O | 29±5 | 115±28 |
| **15b** |  | S | 42±16[c] | 90±7 |
| **15c** |  | O | 22±5[c] | 690±56 |
| **15d** |  | O | 41±5 | 140±1 |
| **15e** |  | O | 15±2 | 690±14 |
| **15f** |  | O | 13±3 | 40±7 |
| **15g** |  | O | 18±3 | 55±7 |
| **15h** |  | O | 28±3 | 835±35 |
| **15i** |  | O | 48±16[c] | >1000 |
| **15j** |  | O | 30±6[c] | 363±10 |
| **15k** |  | S | 27±10[c] | >1000 |
| **15l** |  | S | 35±7[c] | 73±18 |
| **15m** |  | S | 19±2 | 70±7 |
| **15n** |  | S | 12±5[c] | 33±4 |
| **15o** |  | S | 18±2 | 10±1 |
| **15p** |  | S | 19±4[c] | 575±120 |
| **15q** |  | S | 19±2 | 850±14 |

[a] Kinetic constants: $K_M = 22 \pm 3$ μM, $k_{cat} = 4.4\ s^{-1}$; 50 mM Tris·HCl, pH 7.0, 25 °C. [b] Isoniazid $MIC_{50} = 176 \pm 14$ nM; rifampicin $MIC_{50} = 1.7 \pm 0.4$ nM. [c] Mixed model inhibition. Data represent the mean ± SD from two replicate assays for *M. tuberculosis* type II DHQase and three replicate assays for *M. tuberculosis* H37Ra.

nificant inhibition of *M. tuberculosis* growth in vitro ($MIC_{50}$: 10–835 μM). Although there was no clear correlation between the inhibition of *M. tuberculosis* type II DHQase and the $MIC_{50}$ values against *M. tuberculosis* growth, some interesting SAR trends emerged. Firstly, the majority of acetonide-based compounds were at least twofold more potent than the corresponding catechol-based inhibitors against *M. tuberculosis* growth in vitro. This finding may reflect the increased hydrophobicity of the acetonide-based compounds, a factor that has been shown to be important in the penetration of the waxy cell wall of *M. tuberculosis*.[24] It is feasible that the acetonide moiety of these compounds is hydrolyzed after entry into the mycobacterial cell. However, it is entirely possible, given the variation in activity against *M. tuberculosis*, that the compounds prepared in this study inhibit alternate or multiple pathways in vitro. Nonetheless, the incorporation of bicyclic moieties such as benzothiazole and 2-naphthalene groups in **15o** and **15n** resulted in the most potent inhibitors of *M. tuberculosis* growth, with $MIC_{50}$ values of 10 and 33 μM, respectively. Compound **15o** was also shown to possess significant activity against the virulent H37Rv strain of *M. tuberculosis* ($MIC_{50} = 33 \pm 5$ μM) and, although significantly less potent than the frontline TB drugs isoniazid and rifampicin, now serves as a lead for further investigation.

## Conclusions

In summary, we successfully employed an in silico fragment screening approach to identify 3,4-dihydroxyacetophenone and acetonide cores as replacements for the 2,3-anhydroquinate core previously employed in type II DHQase inhibitors. Elaboration of these fragments to incorporate a range of aryl and heteroaryl moieties led to a library of inhibitors that possess low-micromolar inhibition of type II DHQase. Importantly, this study has revealed compounds with promising activity against *M. tuberculosis* growth in vitro. Future work in our research groups will involve co-crystallization of acetophenone and acetonide-based inhibitors with *M. tuberculosis* type II DHQase; this will aid in the rational design of more potent type II DHQase inhibitors. In addition, work is underway to study the molecular mechanism of the antitubercular activity exhibited by these compounds, the results of which will be reported in due course.

## Experimental Section

### *General synthesis procedures*

$^1$H NMR spectra were recorded at 300 K using Bruker Avance DRX200, DRX300, DPX400, or III-600 NMR spectrometers at a frequency of 200.1, 300.2, 400.2, and 600.0 MHz, respectively. $^1$H NMR chemical shifts are reported in parts per million (ppm) and are referenced to solvent residual signals: $CDCl_3$ ($\delta = 7.26$ ppm), MeOD ($\delta = 3.31$ ppm), $(CD_3)_2CO$ ($\delta = 2.05$ ppm). $^1$H NMR data are reported as chemical shift ($\delta_H$), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublet of doublets), coupling constant (*J* in Hz), and assignment where possible. Low-resolution mass spectra were recorded on a Finnigan LCQ Deca ion trap mass spectrometer (ESI). High-resolution mass spectra were recorded on a Bruker 7T Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometer. Melting points were recorded using a Stanford Research Systems OptiMelt Automated Melting Point System. Infrared (IR) absorption spectra were recorded on a Bruker Alpha Spectrometer with attenuated total reflection (ATR) capability and were processed with OPUS 6.5 software.

Analytical reversed-phase HPLC was performed on a Waters System 2695 separations module with an Alliance series column heater at 30 °C and 2996 photodiode array detector. Inhibitors **11** and **16a–q** were analyzed using a Waters Sunfire BEH300 5 μm, 2.1 × 150 mm column ($C_{18}$) at a flow rate of 0.2 mL min$^{-1}$ using a mobile phase of 0.1% TFA in $H_2O$ (Solvent A) and 0.1% TFA in $CH_3CN$ (Solvent B) and a linear gradient of 0 to 100% B over 40 min. Inhibitors **19** and **15a–q** were analyzed with a Waters Sunfire 300 5 μm, 2.1 × 150 mm column ($C_4$) at a flow rate of 0.2 mL min$^{-1}$ using a mobile phase of 0.1% TFA in $H_2O$ (Solvent A) and 0.1% TFA in $CH_3CN$ (Solvent B) and a linear gradient of 20 to 100% B over 40 min. Results were analyzed with Waters Empower software. The purity of the final inhibitors was shown to be > 97% by analytical HPLC.

**Materials**: Analytical thin-layer chromatography (TLC) was performed on commercially prepared silica plates (Merck Kieselgel 60 0.25 mm $F_{254}$). Flash column chromatography was performed using 230–400 mesh Kieselgel 60 silica eluting with distilled solvents as described. Ratios of solvents used for TLC and column chromatography are expressed in *v/v* as specified. Compounds were visualized by UV light at $\lambda$ 254 nm or by using vanillin or cerium molybdate stain. Commercial materials were used as received, unless otherwise noted. Dichloromethane and methanol were distilled from calcium hydride, and THF and diethyl ether were distilled over sodium/benzophenone. Anhydrous DMF was purchased from Sigma–Aldrich.

### *Molecular modeling*

**Protein preparation**: The crystal structures of *M. tuberculosis* type II DHQase were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB code: 2XB8).[19a] The structures were subjected to further modification to ensure suitability for molecular docking using Glide (version 5.0).[21] The ligand–protein co-crystal structures were imported into Accelrys DS visualizer 2.0 (Accelrys Software Inc.) where the dodecamers were simplified into a dimer (Subunits A and B). (2*R*)-2-Methoxybenzyl-3-dehydroquinic acid was maintained in subunit A. Structural water molecules were also removed. The simplified type II DHQase protein structure was then imported into Maestro (version 9.1) and prepared using the *Protein Preparation Wizard* tool, in which bond orders were assigned to the ligands. Hydrogen atoms were added to both ligands and the protein in a manner consistent with physiological pH (pH 7.0) using an all-atom force field. Restrained minimization of the protein structure was then conducted using *impref* using an OPLS-AA force field until the root mean square deviation (RMSD) of non-hydrogen atoms reached 0.3.

**Ligand preparation**: Ligands were built in Maestro (version 9.1) and used as maegz files. The ligands were then subjected to treatment with Ligprep (version 2.3), which generates low-energy 3D structures with tautomers and possible states at pH 7.

**Glide docking**: A receptor grid file was generated using the *Receptor Grid Generation* utility in Glide (version 5.0). The ligand chosen

was (2*R*)-2-methoxybenzyl-3-dehydroquinic acid as the center of receptor grid generation; van der Waals radius scaling and partial charge cutoff default values were used. Per-atom scale factors, which soften receptor potential, were not used. After the receptor grids were generated, ligands were docked into the active site using extra precision (XP) mode.

### *Synthesis*

**1-(3,4-Dihydroxyphenyl)ethanone (11)**: Anhydrous $AlCl_3$ (0.98 g, 7.4 mmol) was dissolved in 1,2-dichloroethane (DCE), and the reaction was cooled to 10 °C for 30 min. Catechol **17** (0.32 g, 2.9 mmol) was added in three portions over 5 min, and the reaction was stirred at 10 °C for a further 30 min. Acetyl chloride (0.26 mL, 3.2 mmol) was added dropwise to the reaction mixture, which was allowed to warm to room temperature and stirred for 20 h. The reaction was subsequently cooled to 10 °C before quenching with 1 M HCl (10 mL), and the reaction mixture was allowed to stir for a further 2 h. The reaction mixture was diluted with $CH_2Cl_2$ (30 mL), and the organic layer was separated. The aqueous layer was extracted with EtOAc (2×30 mL), and the combined organic layers were washed with brine (40 mL) and dried over anhydrous $Na_2SO_4$. The solvent was removed in vacuo to afford acetophenone **11** as a red solid, which was used without further purification (0.43 g, 95%). $R_f$ (8:1 *v/v* $CH_2Cl_2$/$Et_2O$) = 0.30; mp: 120–121 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.47 (1H, dd, *J* = 4.0, 8.0 Hz, Ar-H, C-6), 7.42 (1H, d, *J* = 4.0 Hz, Ar-H, C-2), 6.88 ppm (1H, d, *J* = 8.0 Hz, Ar-H, C-5); $^{13}$C NMR (100 MHz, [$D_6$]acetone): $\delta$ = 195.9, 150.2, 145.3, 130.1, 122.2, 115.6, 114.8, 25.5 ppm; IR (ATR): $\tilde{\nu}$ = 3000, 1796 $cm^{-1}$. These data are in agreement with those previously reported by Xiao et al.[25]

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)ethanone (12)**: A mixture of 3,4-dihydroxyacetophenone **11** (3.87 g, 25 mmol) and $P_2O_5$ (220 mg) in toluene (13 mL) was heated at 75 °C. Acetone (7.4 mL, 100 mmol) was added dropwise to the suspension over 3 h. After the addition, four portions of $P_2O_5$ (4×220 mg) were added to the reaction mixture every 30 min. The reaction was allowed to stir at 75 °C for 5 h. The reaction was quenched with 25% $NaOH_{(aq)}$ (15 mL), and the solvent was removed in vacuo to yield a crude residue that was purified by column chromatography (2:1 *v/v* $CH_2Cl_2$/hexane) to afford acetonide **12** as a pale-yellow oil (1.96 g, 40%). $R_f$ (2:1 *v/v* $CH_2Cl_2$/hexane) = 0.29; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.53 (1H, dd, *J* = 1.6, 8.0 Hz, Ar-H, H-6), 7.35 (1H, s, Ar-H, H-2), 6.75 (1H, d, *J* = 8.0 Hz, Ar-H, H-5), 2.52 (3H, s, $CH_3$), 1.69 (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 196.5, 151.7, 147.9, 131.6, 124.4, 119.4, 107.9, 107.7, 26.5, 25.9 ppm; IR (ATR): $\tilde{\nu}$ = 1675 $cm^{-1}$; MS (ESI): *m/z* 193 $[M+H]^+$.

**2-Bromo-1-(2,2-dimethylbenzo[*d*][1,3]dioxol-5-yl)ethanone (18)**: A solution of acetonide **12** (380 mg, 1.9 mmol) was added to a solution of $CuBr_2$ (830 mg, 3.7 mmol) in EtOAc (2.5 mL) and $CH_2Cl_2$ (2.5 mL). The reaction mixture was held at reflux with vigorous stirring for 9 h. The reaction mixture was then filtered, the solvent removed in vacuo, and the crude residue purified by column chromatography (1:1 *v/v* $CH_2Cl_2$/hexane→2:1 *v/v* $CH_2Cl_2$/hexane) to afford α-bromoketone **18** as a pale-yellow oil (290 mg, 57%). $R_f$ (1:1 *v/v* $CH_2Cl_2$/hexane) = 0.26; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.55 (1H, dd, *J* = 1.8, 8.4 Hz, Ar-H, H-6), 7.36 (1H, d, *J* = 1.8 Hz, Ar-H, H-2), 6.77 (1H, d, *J* = 8.4 Hz, Ar-H, H-5), 4.36 (2H, s, $CH_2$), 1.70 ppm (6H, s, 2×$CH_3$); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 190.0, 152.9, 148.6, 128.5, 125.6, 120.2, 108.8, 108.3, 30.9, 26.3 ppm; IR (ATR): $\tilde{\nu}$ = 1668 $cm^{-1}$.

**General procedure for nucleophilic substitution of bromide 18**: To a suspension of $K_2CO_3$ (0.18–0.23 mmol) in acetone (0.4 mL) was added a suitable aryl or heteroaryl alcohol or thiol (0.22–0.32 mmol), and the reaction mixture was stirred at room temperature for 15 min. Bromide **18** (0.16–0.21 mmol) in acetone (0.4 mL) was added dropwise, and the reaction was stirred at reflux for 4–5 h. The reaction was diluted with $Et_2O$ (10 mL) and washed with $H_2O$ (2×10 mL). The organic layer was dried over anhydrous $MgSO_4$ and the solvent removed in vacuo to yield a crude residue that was purified by column chromatography to afford the corresponding ether or thioether.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-phenoxyethanone (15a)**: Phenol (21 mg, 0.22 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15a** as a pale-yellow oil (40 mg, 67%). $R_f$ (3:2 *v/v* $CH_2Cl_2$/hexane) = 0.45; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.58 (1H, dd, *J* = 1.5, 8.1 Hz, Ar-H), 7.40 (1H, d, *J* = 1.5 Hz, Ar-H), 7.27 (2H, t, *J* = 8.4 Hz, 2×Ar-H), 6.97–6.92 (3H, m, 3×Ar-H), 6.78 (1H, d, *J* = 8.1 Hz, Ar-H), 5.17 (2H, s, $CH_2$), 1.64 (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 192.7, 158.4, 152.9, 148.2, 129.8, 129.1, 124.4, 121.8, 120.1, 115.0, 108.4, 108.2, 70.6, 26.1 ppm; IR (ATR): $\tilde{\nu}$ = 1693 $cm^{-1}$; HRMS calcd for $C_{17}H_{16}O_4Na$ $[M+Na]^+$ 307.0941, found 307.0944.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(phenylthio)ethanone (15b)**: Thiophenol (33 μL, 0.32 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) in acetone (0.4 mL) using the general procedure described above and purified by column chromatography (3:1 *v/v* hexane: $CH_2Cl_2$→3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15b** as a pale-yellow oil (28 mg, 45%). $R_f$ (3:2 *v/v* $CH_2Cl_2$/hexane) = 0.43; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.50 (1H, dd, *J* = 1.5, 8.0 Hz, Ar-H), 7.39 (2H, dd, *J* = 1.4, 7.7 Hz, 2×Ar-H), 7.34 (1H, d, *J* = 1.5 Hz, Ar-H), 7.28 (2H, t, *J* = 7.7 Hz, 2×Ar-H), 7.23–7.20 (1H, m, Ar-H), 6.74 (1H, d, *J* = 8.0 Hz, Ar-H), 4.20 (2H, s, $CH_2$), 1.70 (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 192.5, 152.1, 148.3, 135.1, 131.7, 129.6, 129.1, 127.0, 124.8, 119.7, 108.3, 107.9, 41.9, 22.8 ppm; IR (ATR): $\tilde{\nu}$ = 1671 $cm^{-1}$; HRMS calcd for $C_{17}H_{16}O_3SNa$ $[M+Na]^+$ 323.0712, found 323.0708.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(3-(trifluoromethyl)-phenoxy)ethanone (15c)**: 3-(Trifluoromethyl)phenol (39 μL, 0.32 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15c** as a white solid (67 mg, 90%). $R_f$ (1:1 *v/v* $CH_2Cl_2$/hexane) = 0.24; mp: 115–116 °C; $^1$H NMR (282 MHz, $CDCl_3$): $\delta$ = 7.54 (1H, dd, *J* = 1.8, 8.1 Hz, Ar-H), 7.42–7.33 (2H, m, 2×Ar-H), 7.26–7.20 (1H, m, Ar-H), 7.19–7.14 (1H, m, Ar-H), 7.13–7.04 (1H, m, Ar-H), 6.79 (1H, d, *J* = 8.1 Hz, Ar-H), 5.23 (2H, s, $CH_2$), 1.71 ppm (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 191.9, 158.2, 152.7, 148.3, 131.9 (q, *J* = 33 Hz), 130.2, 128.4, 125.3 (q, *J* = 270 Hz, $CF_3$), 124.2, 119.9, 118.3 (q, *J* = 3.1 Hz), 118.1, 111.9 (q, *J* = 3.2 Hz), 108.1, 107.8, 70.4, 26.0 ppm; IR (ATR): $\tilde{\nu}$ = 1694 $cm^{-1}$; HRMS calcd for $C_{18}H_{15}F_3O_4Na$ $[M+Na]^+$ 375.0815, found 375.0813.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(2-fluorophenoxy)-ethanone (15d)**: 2-Fluorophenol (28 μL, 0.32 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (2:1 *v/v* $CH_2Cl_2$/hexane) to afford **15d** as a white solid (62 mg, 97%). $R_f$ (2:1 *v/v* $CH_2Cl_2$/hexane) = 0.43; mp: 113–114 °C; $^1$H NMR (282 MHz, $CDCl_3$): $\delta$ = 7.57 (1H, dd, *J* = 1.8, 8.4 Hz, Ar-H), 7.38 (1H, d, *J* = 1.8 Hz, Ar-H), 7.08 (1H, dt, *J* = 1.8, 8.1 Hz, Ar-H), 7.00 (1H, dd, *J* = 1.8, 8.1 Hz, Ar-H), 6.95–6.89 (2H, m, 2×Ar-H), 6.78 (1H, d, *J* = 8.4 Hz, Ar-H), 5.25 (2H, s, $CH_2$), 1.70 ppm (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 192.2, 153.3 (d, *J* = 258 Hz, *ipso*-C), 152.5,

148.2, 146.1 (d, $J=9.5$ Hz, *ortho*-C), 128.5, 124.3 (d, $J=4.8$ Hz, *para*-C), 124.2, 122.2 (d, $J=7.9$ Hz, *meta*-C), 119.8, 116.6 (d, $J=19$ Hz, *ortho*-C), 116.0, 108.1, 107.8, 71.9, 26.0 ppm; IR (ATR): $\tilde{\nu}=1692\ cm^{-1}$; HRMS calcd for $C_{17}H_{15}FO_4Na$ $[M+Na]^+$ 325.0845, found 325.0842.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(3-nitrophenoxy)ethanone (15e)**: 3-Nitrophenol (32 mg, 0.23 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (3:1 *v/v* $CH_2Cl_2$/hexane) to afford **15e** as an off-white solid (41 mg, 59%). $R_f$ (3:2 *v/v* $CH_2Cl_2$/hexane) = 0.28; mp: 171–172 °C; $^1$H NMR (300 MHz, 1:1 *v/v* $CDCl_3$/[$D_6$]acetone): $\delta=7.87$ (1H, dd, $J=1.6$, 8.1 Hz, Ar-H), 7.79 (1H, d, $J=1.6$ Hz, Ar-H), 7.64 (1H, dd, $J=1.6$, 8.1 Hz, Ar-H), 7.50 (1H, t, $J=8.1$ Hz, Ar-H), 7.36–7.33 (2H, m, 2×Ar-H), 6.85 (1H, d, $J=8.1$ Hz, Ar-H), 5.46 (2H, s, $CH_2$), 1.74 (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, 1:1 *v/v* $CDCl_3$/[$D_6$]acetone): $\delta=196.5$, 164.0, 157.7, 154.3, 153.4, 135.4, 133.5, 129.2, 127.0, 125.0, 121.4, 114.5, 113.3, 112.7, 75.6, 30.9 ppm; IR (ATR): $\tilde{\nu}=1653\ cm^{-1}$; HRMS calcd for $C_{17}H_{15}NO_6Na$ $[M+Na]^+$ 352.0792, found 352.0793.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(naphthalen-2-yloxy)ethanone (15f)**: 2-Naphthol (31 mg, 0.22 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15f** as a white solid (70 mg, 91%). $R_f$ (3:2 *v/v* $CH_2Cl_2$/hexane) = 0.45; mp: 129–130 °C (dec.); $^1$H NMR (300 MHz, $CDCl_3$): $\delta=7.77$–7.75 (2H, m, 2×Ar-H), 7.71 (1H, d, $J=8.4$ Hz, Ar-H), 7.63 (1H, dd, $J=1.6$, 8.0 Hz, Ar-H), 7.45–7.43 (2H, m, 2×Ar-H), 7.34 (1H, t, $J=7.2$ Hz, Ar-H), 7.26 (1H, dd, $J=2.4$, 8.8 Hz, Ar-H), 7.11 (1H, d, $J=2.4$ Hz, Ar-H), 6.80 (1H, d, $J=8.0$ Hz, Ar-H), 5.30 (2H, s, $CH_2$), 1.72 ppm (6H, s, 2×$CH_3$); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta=192.9$, 156.5, 152.8, 148.6, 134.7, 130.1, 129.8, 129.3, 128.1, 127.3, 126.9, 124.6, 124.4, 120.0, 119.1, 108.4, 108.3, 107.7, 71.1, 26.3 ppm; IR (ATR): $\tilde{\nu}=1689\ cm^{-1}$; HRMS calcd for $C_{21}H_{18}O_4Na$ $[M+Na]^+$ 357.1097, found 357.1091.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(naphthalen-1-yloxy)ethanone (15g)**: 1-Naphthol (31 mg, 0.22 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15g** as a colorless oil (54 mg, 77%). $R_f$ (3:2 *v/v* $CH_2Cl_2$/hexane) = 0.51; $^1$H NMR (300 MHz, $CDCl_3$): $\delta=8.40$–8.37 (1H, m, Ar-H), 7.83–7.79 (1H, m, Ar-H), 7.65 (1H, dd, $J=1.5$, 8.1 Hz, Ar-H), 7.52–7.46 (4H, m, 4×Ar-H), 7.32 (1H, t, $J=8.1$ Hz, Ar-H), 6.80–6.75 (2H, m, 2×Ar-H), 5.33 (2H, s, $CH_2$), 1.71 (6H, s, 2×$CH_3$); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta=193.1$, 154.3, 152.7, 148.6, 135.0, 129.3, 127.8, 126.9, 126.1, 126.0, 125.9, 124.8, 122.6, 121.6, 120.0, 108.3, 105.7, 71.6, 26.3 ppm; IR (ATR): $\tilde{\nu}=1698\ cm^{-1}$; HRMS calcd for $C_{21}H_{19}O_4$ $[M+H]^+$ 335.1278, found 335.1280.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(2-(isoxazol-5-yl)phenoxy)ethanone (15h)**: 2-(5-Isoxazolyl)phenol (38 mg, 0.23 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (4:1 *v/v* $CH_2Cl_2$/hexane) to afford **15h** as an off-white solid (38 mg, 52%). $R_f$ (4:1 *v/v* $CH_2Cl_2$/hexane) = 0.32; mp: 138–139 °C (dec.); $^1$H NMR (300 MHz, 1:1 *v/v* $CDCl_3$/[$D_6$]acetone): $\delta=8.38$ (1H, s, Ar-H), 7.98 (1H, d, $J=8.0$ Hz, Ar-H), 7.68–7.65 (1H, m, Ar-H), 7.48 (1H, s, Ar-H), 7.42–7.38 (2H, m, 2×Ar-H), 7.15 (1H, d, $J=8.0$ Hz, Ar-H), 7.10 (1H, t, $J=8.0$ Hz, Ar-H), 6.82 (1H, d, $J=8.0$ Hz, Ar-H), 5.52 (2H, s, $CH_2$), 1.71 (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, 1:1 *v/v* $CDCl_3$/[$D_6$]acetone): $\delta=192.0$, 154.6, 152.3, 151.2, 148.2, 131.1, 128.6, 127.4, 123.9, 121.4, 119.7, 116.7, 112.6, 108.0, 107.3, 103.9, 70.4, 25.6 ppm; IR (ATR): $\tilde{\nu}=1697\ cm^{-1}$; HRMS calcd for $C_{20}H_{17}NO_5Na$ $[M+Na]^+$ 374.0998, found 374.1002.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(4-(naphthalen-2-yl)phenoxy)ethanone (15i)**: 4-(Naphthalen-2-yl)phenol (40 mg, 0.18 mmol) was reacted with bromide **18** (45 mg, 0.16 mmol) using the general procedure described above and purified by column chromatography (3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15i** as a white solid (40 mg, 62%). $R_f$ (2:1 *v/v* $CH_2Cl_2$/hexane) = 0.40; mp: 119–120 °C (dec.) $^1$H NMR (400 MHz, $CDCl_3$): $\delta=7.99$ (1H, s, Ar-H), 7.90–7.84 (3H, m, 3×Ar-H), 7.70 (1H, d, $J=8.3$ Hz, Ar-H), 7.66–7.61 (3H, m, 3×Ar-H), 7.51–7.44 (3H, m, 3×Ar-H), 7.05 (2H, d, $J=8.4$ Hz, 2×Ar-H), 6.81 (1H, d, $J=8.1$ Hz, Ar-H), 5.24 (2H, s, $CH_2$), 1.72 ppm (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta=192.5$, 157.8, 152.3, 148.2, 138.0, 134.4, 132.5, 128.8, 128.5, 128.4, 128.0, 127.6, 126.2, 125.7, 125.4, 125.1, 124.2, 119.7, 115.2, 108.0, 107.9, 70.6, 25.7 ppm; IR (ATR): $\tilde{\nu}=1691\ cm^{-1}$; HRMS calcd for $C_{27}H_{23}O_4$ $[M+H]^+$ 411.1596, found 411.1591.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(3-(naphthalen-2-yl)phenoxy)ethanone (15j)**: 3-(Naphthalen-2-yl)phenol (40 mg, 0.18 mmol) was reacted with bromide **18** (45 mg, 0.16 mmol) using the general procedure described above and purified by column chromatography (3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15j** as a foam (38 mg, 58%). $R_f$ (2:1 *v/v* $CH_2Cl_2$/hexane) = 0.50; $^1$H NMR (400 MHz, $CDCl_3$): $\delta=8.02$ (1H, d, $J=1.4$ Hz, Ar-H), 7.92–7.85 (3H, m, 3×Ar-H), 7.72 (1H, dd, $J=1.8$, 8.5 Hz, Ar-H), 7.62 (1H, dd, $J=1.8$, 8.2 Hz, Ar-H), 7.51–7.48 (2H, m, 2×Ar-H), 7.44 (1H, d, $J=1.8$ Hz, Ar-H), 7.40 (1H, app.t, $J=7.8$ Hz, Ar-H), 7.36 (1H, app.t, $J=1.4$ Hz, Ar-H), 7.34–7.32 (1H, m, Ar-H), 6.94 (1H, ddd, $J=1.4$, 2.6, 7.8 Hz, Ar-H), 6.80 (1H, d, $J=8.2$ Hz, Ar-H), 5.26 (2H, s, $CH_2$), 1.73 ppm (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta=192.6$, 158.9, 152.2, 147.9, 142.7, 137.9, 133.6, 132.7, 129.9, 128.8, 128.4, 128.2, 127.6, 126.3, 126.0, 125.9, 125.6, 124.7, 120.8, 119.7, 114.3, 113.3, 108.0, 107.9, 70.7, 26.3 ppm; IR (ATR): $\tilde{\nu}=1706\ cm^{-1}$; HRMS calcd for $C_{27}H_{23}O_4$ $[M+H]^+$ 411.1596, found 411.1591.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(2-(trifluoromethyl)phenylthio)ethanone (15k)**: 2-Trifluoromethyl benzenethiol (43 μL, 0.32 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15k** as a pale-yellow oil (38 mg, 49%). $R_f$ (3:2 *v/v* $CH_2Cl_2$/hexane) = 0.79; $^1$H NMR (400 MHz, $CDCl_3$): $\delta=7.65$ (1H, m, Ar-H), 7.59–7.50 (2H, m, 2×Ar-H), 7.46 (1H, t, $J=7.6$ Hz, Ar-H), 7.35–7.31 (2H, m, 2×Ar-H), 6.76 (1H, d, $J=8.0$ Hz, Ar-H), 4.24 (2H, s, $CH_2$), 1.07 (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta=192.2$, 152.6, 148.4, 134.7, 133.4, 132.6, 130.8 (q, $J=30$ Hz), 129.8, 127.4, 127.2 (q, $J=5.5$ Hz), 125.4 (q, $J=272$ Hz, $CF_3$), 125.2, 120.0, 108.6, 108.2, 41.4, 26.0 ppm; IR (ATR): $\tilde{\nu}=1672\ cm^{-1}$; HRMS calcd for $C_{18}H_{15}F_3O_3SNa$ $[M+Na]^+$ 391.0586, found 391.0588.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-((4-fluorophenyl)thio)ethanone (15l)**: 4-Fluorothiophenol (34 μL, 0.32 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15l** as a pale-yellow oil (42 mg, 63%). $R_f$ (3:2 *v/v* $CH_2Cl_2$/hexane) = 0.28; $^1$H NMR (300 MHz, $CDCl_3$): $\delta=7.47$ (1H, dd, $J=1.8$, 8.1 Hz, Ar-H), 7.45–7.36 (2H, m, 2×Ar-H), 7.31 (1H, d, $J=1.8$ Hz, Ar-H), 7.00–6.94 (2H, m, 2×Ar-H), 6.75 (1H, d, $J=8.1$ Hz, Ar-H), 4.08 (2H, s, $CH_2$), 1.72 ppm (6H, s, 2×$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta=192.6$, 162.0 (d, $J=210$ Hz, *ipso*-C), 152.4, 148.6, 134.1 (d, $J=8.1$ Hz, *meta*-C), 130.0 (d, $J=3.1$ Hz, *para*-C), 129.8, 125.1, 120.0, 116.5 (d, $J=22$ Hz, *ortho*-C), 108.6, 108.2,

42.0, 26.0 ppm; IR (ATR): $\tilde{\nu}=1649\ cm^{-1}$; HRMS calcd for $C_{17}H_{15}FO_3SNa$ $[M+Na]^+$ 341.0618, found 341.0615.

**2-((4-Chlorophenyl)thio)-1-(2,2-dimethylbenzo[*d*][1,3]dioxol-5-yl)ethanone (15m)**: 4-Chlorothiophenol (45 mg, 0.32 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15m** as a pale-yellow oil (31 mg, 44%). $R_f$ (3:2 *v/v* $CH_2Cl_2$/hexane) = 0.59; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta=7.50$ (1H, dd, $J=1.5$, 8.1 Hz, Ar-H), 7.33 (2H, d, $J=8.8$ Hz, 2×Ar-H), 7.26–7.23 (3H, m, 3×Ar-H), 6.75 (1H, d, $J=8.1$ Hz, Ar-H), 4.17 (2H, s, $CH_2$), 1.70 ppm (6H, s, 2×$CH_3$); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta=192.4$, 152.6, 148.5, 133.9, 133.5, 132.2, 129.9, 129.6, 125.1, 120.0, 108.6, 108.2, 41.4, 26.3 ppm; IR (ATR): $\tilde{\nu}=1672\ cm^{-1}$; HRMS calcd for $C_{17}H_{16}ClO_3S$ $[M+H]^+$ 335.0503, found 335.0502.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-(naphthalen-2-ylthio)ethanone (15n)**: 2-Naphthalenethiol (51 mg, 0.31 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above and purified by column chromatography (3:2 *v/v* $CH_2Cl_2$/hexane) to afford **15n** as a pale-yellow oil (39 mg, 53%). $R_f$ (2:1 *v/v* $CH_2Cl_2$/hexane) = 0.53; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta=7.83$–7.73 (4H, m, 4×Ar-H), 7.55 (1H, dd, $J=1.8$, 8.1 Hz, Ar-H), 7.48 (1H, d, $J=1.8$ Hz, Ar-H), 7.47–7.43 (2H, m, 2×Ar-H), 7.36 (1H, d, $J=1.5$ Hz, Ar-H), 6.75 (1H, d, $J=8.1$ Hz, Ar-H), 4.30 (2H, s, $CH_2$), 1.69 ppm (6H, s, 2×$CH_3$); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta=192.7$, 152.5, 148.5, 134.1, 132.9, 132.6, 130.1, 129.0, 128.3, 128.1, 127.7, 126.9, 126.4, 125.1, 119.9, 108.7, 108.2, 41.3, 26.3 ppm; IR (ATR): $\tilde{\nu}=1668\ cm^{-1}$; HRMS calcd for $C_{21}H_{18}O_3SNa$ $[M+Na]^+$ 373.0869, found 373.0863.

**2-(Benzo[*d*]thiazol-2-ylthio)-1-(2,2-dimethylbenzo[*d*][1,3]dioxol-5-yl)ethanone (15o)**: 2-Mercaptobenzothiazole (66 mg, 0.36 mmol) was reacted with bromide **18** (50 mg, 0.18 mmol) using the general procedure described above and purified by column chromatography (49:1 *v/v* $CH_2Cl_2$: acetone) to afford **15o** as a pale-yellow oil (39 mg, 61%). $R_f$ (2:1 *v/v* $CH_2Cl_2$/hexane) = 0.20; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta=7.83$ (1H, app.d, $J=8.0$ Hz, Ar-H), 7.74 (1H, app.d, $J=7.8$ Hz, Ar-H), 7.67 (1H, app.d, $J=7.5$ Hz, Ar-H), 7.44–7.38 (2H, m, 2×Ar-H), 7.31–7.26 (1H, m, Ar-H), 6.80 (1H, d, $J=8.0$ Hz, Ar-H), 4.87 (2H, s, $CH_2$), 1.71 ppm (6H, s, 2×$CH_3$); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta=190.7$, 164.8, 152.5, 151.9, 147.4, 135.0, 129.2, 125.6, 124.8, 123.8, 121.1, 120.8, 119.2, 107.7, 107.4, 40.3, 25.5 ppm; IR (ATR): $\tilde{\nu}=1673\ cm^{-1}$; HRMS calcd for $C_{18}H_{16}NO_3S_2$ $[M+H]^+$ 358.0566, found 358.0569.

**1-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-((4,5-diphenyloxazol-2-yl)thio)ethanone (15p)**: 4,5-Diphenyloxazole-2-thiol (61 mg, 0.24 mmol) was reacted with bromide **18** (50 mg, 0.18 mmol) using the general procedure described above and purified by column chromatography (2:1 *v/v* $CH_2Cl_2$/hexane) to afford **15p** as a colorless oil (52 mg, 65%). $R_f$ (2:1 *v/v* $CH_2Cl_2$/hexane) = 0.29; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta=7.65$ (1H, dd, $J=1.6$, 8.0 Hz, Ar-H), 7.62 (2H, dd, $J=1.6$, 8.0 Hz, 2×Ar-H), 7.54 (2H, dd, $J=1.6$, 8.0 Hz, 2×Ar-H), 7.44 (1H, d, $J=1.6$ Hz, Ar-H), 7.39–7.31 (6H, m, Ar-H), 6.79 (1H, d, $J=8.0$ Hz, Ar-H), 4.77 (2H, s, $CH_2$), 1.71 ppm (6H, s, 2×$CH_3$); $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta=191.1$, 158.3, 152.5, 148.2, 147.4, 136.4, 129.6, 128.7, 128.6, 128.5, 126.4, 124.8, 119.7, 108.1, 107.8, 40.6, 25.6 ppm; IR (ATR): $\tilde{\nu}=1675\ cm^{-1}$; HRMS calcd for $C_{26}H_{22}NO_4S$ $[M+H]^+$ 444.1264, found 444.1264.

**2-((2-(2,2-Dimethylbenzo[*d*][1,3]dioxol-5-yl)-2-oxoethyl)thio)quinazolin-4(3*H*)-one (15q)**: 2-Mercaptoquinazolin-4(3*H*)-one (56 mg, 0.31 mmol) was reacted with bromide **18** (57 mg, 0.21 mmol) using the general procedure described above to yield **15q** as a white solid which was used without further purification (55 mg, 71%). $R_f$ (9:1 *v/v* $CH_2Cl_2$/acetone) = 0.54; mp: 219–220 °C (dec.); $^1H$ NMR (400 MHz, $[D_6]$DMSO): $\delta=8.04$ (1H, dd, $J=1.2$, 7.9 Hz, Ar-H), 7.76 (1H, dd, $J=1.7$, 8.2 Hz, Ar-H), 7.72 (1H, dt, $J=1.5$, 8.5 Hz, Ar-H), 7.50 (1H, d, $J=1.7$ Hz, Ar-H), 7.42 (1H, dt, $J=1.2$, 7.9 Hz), 7.27 (1H, app.d, $J=8.5$ Hz, Ar-H), 7.05 (1H, d, $J=8.2$ Hz, Ar-H), 4.82 (2H, s, $CH_2$), 1.73 ppm (6H, s, 2×$CH_3$); $^{13}C$ NMR (100 MHz, $[D_6]$DMSO): $\delta=192.0$, 161.6, 155.6, 151.9, 148.4, 147.4, 134.9, 130.7, 126.6, 126.1, 125.0, 120.3, 120.1, 108.5, 108.1, 37.8, 26.0 ppm; IR (ATR): $\tilde{\nu}=1661\ cm^{-1}$; HRMS calcd for $C_{19}H_{17}N_2O_4S$ $[M+H]^+$ 369.0904, found 369.0903.

**General procedure for the deprotection of 15a–q**: Aqueous trifluoroacetic acid (90%, 0.3–0.4 mL) was added dropwise to acetonide-based inhibitor **15a–q** (0.017–0.12 mmol), and the reaction was stirred at room temperature or at 50 °C for 1.5–5 h. At this point, the solvent was removed in vacuo to yield a crude residue that was purified by column chromatography.

**1-(3,4-Dihydroxyphenyl)-2-phenoxyethanone (16a)**: Acetonide-based inhibitor **15a** (27 mg, 0.095 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (95:5 *v/v* $CH_2Cl_2$/$CH_3OH$) to afford inhibitor **16a** as an off-white solid (20 mg, 87%). $R_f$ (95:5 *v/v* $CH_2Cl_2$/$CH_3OH$) = 0.26; mp: 157–158 °C (dec.); $^1H$ NMR (400 MHz, $[D_6]$acetone): $\delta=7.55$–7.57 (2H, m, 2×Ar-H), 7.25–7.29 (2H, m, 2×Ar-H), 6.91–6.96 (4H, m, 4×Ar-H), 5.38 ppm (2H, s, $CH_2$); $^{13}C$ NMR (100 MHz, $[D_6]$acetone): $\delta=192.7$, 159.0, 150.9, 145.5, 129.7, 128.0, 122.0, 121.2, 115.3, 115.1, 115.0, 70.4 ppm; IR (ATR): $\tilde{\nu}=3404$ (br. OH str.), 3367 (br. OH str.), 1676 $cm^{-1}$; HRMS calcd for $C_{14}H_{12}O_4Na$ $[M+Na]^+$ 267.0628, found 267.0624.

**1-(3,4-Dihydroxyphenyl)-2-(phenylthio)ethanone (16b)**: Acetonide-based inhibitor **15b** (25 mg, 0.083 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (98:2 *v/v* $CH_2Cl_2$/$CH_3OH$) to afford inhibitor **16b** as a pale-yellow oil (23 mg, 93%). $R_f$ (9:1 *v/v* $CH_2Cl_2$/$CH_3OH$) = 0.43; $^1H$ NMR (300 MHz, $[D_6]$acetone): $\delta=7.53$–7.50 (2H, m, 2×Ar-H), 7.41–7.39 (2H, dd, $J=1.2$, 7.5 Hz, 2×Ar-H), 7.29 (2H, t, $J=7.5$ Hz, 2×Ar-H), 7.19 (1H, t, $J=7.5$ Hz, Ar-H), 6.92 (1H, d, $J=8.4$ Hz, Ar-H), 4.41 ppm (2H, s, $CH_2$); $^{13}C$ NMR (100 MHz, $[D_6]$acetone): $\delta=192.5$, 150.9, 145.4, 136.6, 129.4, 129.3, 128.7, 126.5, 122.8, 115.7, 115.2, 40.3 ppm; IR (ATR): $\tilde{\nu}=3474$ (br. OH str.), 3270 (br. OH str.), 1657 $cm^{-1}$; HRMS calcd for $C_{14}H_{12}SO_3Na$ $[M+Na]^+$ 283.0399, found 283.0395.

**1-(3,4-Dihydroxyphenyl)-2-(3-(trifluoromethyl)phenoxy)ethanone (16c)**: Acetonide-based inhibitor **15c** (20 mg, 0.057 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (95:5 *v/v* $CH_2Cl_2$/$CH_3OH$) to afford inhibitor **16c** as a white solid (14 mg, 78%). $R_f$ (95:5 *v/v* $CH_2Cl_2$/$CH_3OH$) = 0.32; mp: 192–193 °C (dec.); $^1H$ NMR (282 MHz, $[D_6]$acetone): $\delta=7.56$–7.49 (3H, m, Ar-H), 7.29–7.24 (3H, m, Ar-H), 6.95 (2H, d, $J=8.0$ Hz, 2×Ar-H), 5.56 ppm (2H, s, $CH_2$); $^{13}C$ NMR (100 MHz, $[D_6]$acetone): $\delta=191.1$, 158.6, 150.4, 144.6, 130.6 (q, $J=32$ Hz), 129.9, 126.9, 122.4 (q, $J=262$ Hz, $CF_3$), 121.1, 118.1, 117.5 (app.d, $J=3.7$ Hz), 114.5, 114.2, 111.0 (app.d, $J=3.4$ Hz), 69.6 ppm; IR (ATR): $\tilde{\nu}=3508$ (br. OH str.), 3349 (br. OH str.), 1678 $cm^{-1}$; HRMS calcd for $C_{15}H_{11}F_3O_4Na$ $[M+Na]^+$ 335.0502, found 335.0501.

**1-(3,4-Dihydroxyphenyl)-2-(2-fluorophenoxy)ethanone (16d)**: Acetonide-based inhibitor **15d** (17 mg, 0.056 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (98:2 *v/v* $CH_2Cl_2$/$CH_3OH$) to afford inhibitor **16d** as a white solid (14 mg,

98%). $R_f$ (98:2 *v/v* $CH_2Cl_2/CH_3OH$) = 0.20; mp: 190–191 °C (dec.); $^1$H NMR (282 MHz, [D$_6$]acetone): $\delta$ = 7.54–7.51 (2H, m, Ar-H), 7.13 (1H, t, $J$ = 7.8 Hz, Ar-H), 7.06–7.03 (2H, m, 2×Ar-H), 6.94–6.92 (2H, m, 2×Ar-H), 5.46 ppm (2H, s, $CH_2$); $^{13}$C NMR (75 Hz, [D$_6$]acetone): $\delta$ = 193.1, 153.7 (d, $J$ = 244 Hz, *ipso*-C), 152.1, 147.9 (d, $J$ = 11 Hz, *ortho*-C), 146.4, 128.6, 125.6 (d, $J$ = 3.8 Hz, *para*-C), 122.9, 122.6 (d, $J$ = 6.8 Hz, *meta*-C), 117.2 (d, $J$ = 18 Hz, *ortho*-C), 116.5, 116.2, 115.7, 71.9 ppm; IR (ATR): $\tilde{\nu}$ = 3320 (br. OH str.), 1691 cm$^{-1}$; HRMS calcd for $C_{14}H_{11}FO_4Na$ $[M+Na]^+$ 285.0534, found 285.0537.

**1-(3,4-Dihydroxyphenyl)-2-(3-nitrophenoxy)ethanone (16e)**: Acetonide-based inhibitor **15e** (22 mg, 0.067 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (95:5 *v/v* $CH_2Cl_2$/$CH_3OH$) to afford inhibitor **16e** as an off-white solid (16 mg, 85%). $R_f$ (95:5 *v/v* $CH_2Cl_2/CH_3OH$) = 0.21; mp: 206–207 °C (dec.); $^1$H NMR (300 MHz, [D$_6$]acetone): $\delta$ = 7.83 (1H, dd, $J$ = 1.2, 8.1 Hz, Ar-H), 7.81 (1H, d, $J$ = 2.7 Hz, Ar-H), 7.60–7.54 (3H, m, 3×Ar-H), 7.41 (1H, dd, $J$ = 1.6, 8.0 Hz, Ar-H), 6.95 (1H, d, $J$ = 8.0 Hz, Ar-H), 5.62 ppm (2H, s, $CH_2$); $^{13}$C NMR (75 MHz, [D$_6$]acetone): $\delta$ = 192.6, 160.6, 152.2, 150.5, 146.5, 131.6, 128.5, 123.0, 122.9, 116.9, 116.3, 115.9, 110.5, 71.6 ppm; IR (ATR): $\tilde{\nu}$ = 3491 (br. OH str.), 3398 (br. OH str.), 1679 cm$^{-1}$; HRMS calcd for $C_{14}H_{11}NO_6Na$ $[M+Na]^+$ 312.0479, found 312.0476.

**1-(3,4-Dihydroxyphenyl)-2-(naphthalen-2-yloxy)ethanone (16f)**: Acetonide-based inhibitor **15f** (39 mg, 0.12 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (95:5 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16f** as an off-white solid (31 mg, 88%). $R_f$ (95:5 *v/v* $CH_2Cl_2/CH_3OH$) = 0.36; mp: 174–175 °C (dec.); $^1$H NMR (300 MHz, [D$_6$]acetone): $\delta$ = 7.86–7.84 (2H, m, 2×Ar-H), 7.75 (1H, d, Ar-H, $J$ = 8.1 Hz), 7.62–7.60 (2H, m, 2×Ar-H), 7.43 (1H, dt, $J$ = 1.5, 8.1 Hz, Ar-H), 7.36–7.32 (2H, m, 2×Ar-H), 7.25 (1H, dd, $J$ = 2.4, 9.0 Hz, Ar-H), 6.98 (1H, d, $J$ = 8.7 Hz, Ar-H), 5.51 (2H, s, $CH_2$); $^{13}$C NMR (100 MHz, [D$_6$]acetone): $\delta$ = 191.6, 156.1, 150.2, 144.6, 134.2, 128.9, 128.8, 127.2, 127.1, 126.3, 125.8, 123.2, 121.2, 118.2, 114.5, 114.4, 106.8, 69.7 ppm); IR (ATR): $\tilde{\nu}$ = 3423 (br. OH str.), 3283 (br. OH str.), 1682 cm$^{-1}$; HRMS calcd for $C_{18}H_{14}O_4Na$ $[M+Na]^+$ 317.0784, found 317.0787.

**1-(3,4-Dihydroxyphenyl)-2-(naphthalen-1-yloxy)ethanone (16g)**: Acetonide-based inhibitor **15g** (39 mg, 0.12 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (98:2 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16g** as a colorless oil (31 mg, 88%). $R_f$ (9:1 *v/v* $CH_2Cl_2/CH_3OH$) = 0.51; $^1$H NMR (300 MHz, [D$_6$]acetone): $\delta$ = 8.40–8.37 (1H, m, Ar-H), 7.88–7.85 (1H, m, Ar-H), 7.63–7.61 (2H, m, 2×Ar-H), 7.55–7.47 (3H, m, 3×Ar-H), 7.38 (1H, t, $J$ = 7.8 Hz, Ar-H), 6.99–6.94 (2H, m, 2×Ar-H), 5.57 ppm (2H, s, $CH_2$); $^{13}$C NMR (75 MHz, [D$_6$]acetone): $\delta$ = 192.4, 154.5, 151.2, 145.6, 135.1, 127.9, 127.8, 126.7, 126.2, 126.1, 125.5, 122.6, 122.5, 120.8, 115.5, 115.3, 105.8, 70.8 ppm; IR (ATR): $\tilde{\nu}$ = 3518 (br. OH str.), 3430 (br. OH str.), 1705 cm$^{-1}$; HRMS calcd for $C_{18}H_{14}O_4Na$ $[M+Na]^+$ 317.0784, found 317.0787.

**1-(3,4-Dihydroxyphenyl)-2-(2-(isoxazol-5-yl)phenoxy)ethanone (16h)**: Acetonide-based inhibitor **15h** (29 mg, 0.082 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (9:1 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16h** as an off-white solid (23 mg, 92%). $R_f$ (9:1 $CH_2Cl_2/CH_3OH$) = 0.50; mp: 198–199 °C (dec.); $^1$H NMR (300 MHz, [D$_6$]acetone): $\delta$ = 8.53 (1H, d, $J$ = 1.8 Hz, Ar-H), 8.03 (1H, dd, $J$ = 1.5, 7.8 Hz, Ar-H), 7.66–7.64 (2H, m, 2×Ar-H), 7.56 (1H, d, $J$ = 1.8 Hz, Ar-H), 7.50 (1H, t, $J$ = 8.4 Hz, Ar-H), 7.33 (1H, d, $J$ = 8.4 Hz, Ar-H), 7.19 (1H, t, $J$ = 7.8 Hz), 7.00 (1H, d, $J$ = 8.7 Hz, Ar-H), 5.69 ppm (2H, s, $CH_2$); $^{13}$C NMR (100 MHz, [D$_6$]acetone): $\delta$ = 191.3, 164.8, 154.9, 151.2, 150.9, 145.3, 131.2, 127.2, 127.1, 121.6, 121.2, 116.5, 115.1, 114.6, 112.9, 104.3, 70.4 ppm; IR (ATR): $\tilde{\nu}$ = 3352 (br. OH str.), 3234 (br. OH str.), 1683 cm$^{-1}$; HRMS calcd for $C_{17}H_{13}NO_5Na$ $[M+Na]^+$ 334.0686, found 334.0689.

**1-(3,4-Dihydroxyphenyl)-2-(4-(naphthalen-2-yl)phenoxy)ethanone (16i)**: Acetonide-based inhibitor **15i** (10 mg, 0.024 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (9:1 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16i** as a foam (7.5 mg, 84%). $R_f$ (9:1 *v/v* $CH_2Cl_2/CH_3OH$) = 0.56; $^1$H NMR (400 MHz, [D$_6$]acetone): $\delta$ = 8.13 (1H, s, Ar-H), 7.98–7.96 (2H, m, 2×Ar-H), 7.91 (1H, d, $J$ = 8.4 Hz, Ar-H), 7.81 (1H, dd, $J$ = 1.8, 8.6 Hz, Ar-H), 7.75 (2H, d, $J$ = 8.8 Hz, 2×Ar-H), 7.60–7.58 (2H, m, 2×Ar-H), 7.54–7.47 (2H, m, 2×Ar-H), 7.11 (2H, d, $J$ = 8.8 Hz, 2×Ar-H), 6.97 (1H, d, $J$ = 8.0 Hz, Ar-H), 5.46 ppm (2H, s, $CH_2$); $^{13}$C NMR (100 MHz, [D$_6$]acetone): $\delta$ = 192.2, 158.3, 150.9, 145.2, 137.9, 133.9, 133.4, 132.4, 128.3, 128.1, 128.0, 127.5, 126.2, 125.6, 125.1, 124.7, 121.6, 115.1, 114.9, 114.7, 70.0 ppm; IR (ATR): $\tilde{\nu}$ = 3300 (br. OH str.), 1726 cm$^{-1}$; HRMS calcd for $C_{24}H_{19}O_4$ $[M+H]^+$ 371.1278, found 371.1278.

**1-(3,4-Dihydroxyphenyl)-2-(3-(naphthalen-2-yl)phenoxy)ethanone (16j)**: Acetonide-based inhibitor **15j** (21 mg, 0.051 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (9:1 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16j** as a white foam (16 mg, 84%). $R_f$ (9:1 *v/v* $CH_2Cl_2/CH_3OH$) = 0.50; $^1$H NMR (400 MHz, [D$_6$]acetone): $\delta$ = 8.17 (1H, s, Ar-H), 7.99–7.96 (2H, m, 2×Ar-H), 7.92 (1H, dd, $J$ = 2.1 Hz, 8.8 Hz, Ar-H), 7.81 (1H, dd, $J$ = 1.8, 8.5 Hz, Ar-H), 7.66–7.56 (2H, m, 2×Ar-H), 7.54–7.50 (2H, m, 2×Ar-H), 7.41–7.39 (3H, m, 3×Ar-H), 7.00 (1H, ddd, $J$ = 1.7, 2.4, 7.6 Hz, Ar-H), 6.95 (1H, d, $J$ = 8.2 Hz), 5.49 ppm (2H, s, 2×$CH_2$); $^{13}$C NMR (100 MHz, [D$_6$]acetone): $\delta$ = 192.0, 159.2, 150.9, 145.1, 142.1, 138.0, 133.7, 132.9, 129.9, 128.4, 128.2, 127.5 (×2) 126.2, 126.0, 125.6, 125.2, 121.7, 119.9, 114.9, 114.7, 113.7, 113.5, 69.8 ppm; IR (ATR): $\tilde{\nu}$ = 3339 (br. OH), 1689 cm$^{-1}$; HRMS calcd for $C_{24}H_{19}O_4$ $[M+H]^+$ 371.1278, found 371.1273.

**1-(3,4-Dihydroxyphenyl)-2-((2-(trifluoromethyl)phenyl)thio)ethanone (16k)**: Acetonide-based inhibitor **15k** (18 mg, 0.049 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (98:2 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16k** as an off-white solid (13 mg, 86%). $R_f$ (9:1 *v/v* $CH_2Cl_2/CH_3OH$) = 0.45; mp: 169–170 °C (dec.); $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ = 7.68 (1H, dd, $J$ = 1.2, 7.8 Hz, Ar-H), 7.62 (1H, dd, $J$ = 1.2, 7.8 Hz, Ar-H), 7.53 (1H, dt, $J$ = 1.5, 7.8 Hz, Ar-H), 7.48–7.43 (2H, m, 2×Ar-H), 7.35 (1H, dt, $J$ = 1.2, 7.5 Hz, Ar-H), 6.82 (1H, d, $J$ = 8.5 Hz, Ar-H), 4.87 ppm (2H, s, $CH_2$); $^{13}$C NMR (100 MHz, [D$_6$]acetone): $\delta$ = 191.9, 151.1, 145.4, 136.4, 132.9, 131.2, 128.9, 128.6, 127.0 (q, $J$ = 28 Hz), 126.3, 125.9 (q, $J$ = 271 Hz, $CF_3$), 122.9, 115.6, 115.3, 40.7 ppm; IR (ATR): $\tilde{\nu}$ = 3485 (br. OH str.), 3367 (br. OH str.), 1657 cm$^{-1}$; HRMS calcd for $C_{15}H_{12}F_3O_3S$ $[M+H]^+$ 329.0454, found 329.0454.

**1-(3,4-Dihydroxyphenyl)-2-((4-fluorophenyl)thio)ethanone (16l)**: Acetonide-based inhibitor **15l** (29 mg, 0.091 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (98:2 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16l** as a pale-yellow oil (18 mg, 73%). $R_f$ (9:1 *v/v* $CH_2Cl_2/CH_3OH$) = 0.42; $^1$H NMR (300 MHz, [D$_6$]acetone): $\delta$ = 7.58–7.42 (4H, m, 4×Ar-H), 7.09–7.04 (2H, m, 2×Ar-H), 6.78 (1H, d, $J$ = 7.8 Hz, Ar-H), 4.35 ppm (2H, s, $CH_2$); $^{13}$C NMR

(100 MHz, [D$_6$]acetone): $\delta$ = 192.1, 162.0 (d, $J$ = 240 Hz, *ipso*-C), 150.6, 145.0, 132.4 (d, $J$ = 8.0 Hz, *meta*-C), 131.3 (d, $J$ = 3.1 Hz, *para*-C), 128.2, 122.5, 116.0 (d, $J$ = 20 Hz, *ortho*-C), 115.3, 114.7, 40.9 ppm; IR (ATR): $\tilde{\nu}$ = 3527 (br. OH str.), 3329 (br. OH str.), 1654 cm$^{-1}$; HRMS calcd for $C_{14}H_{11}FO_3SNa$ $[M+Na]^+$ 301.0305, found 301.0301.

**2-((4-Chlorophenyl)thio)-1-(3,4-dihydroxyphenyl)ethanone (16m)**: Acetonide-based inhibitor **15m** (20 mg, 0.060 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (98:2 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16m** as a pale-yellow oil (17 mg, 98%). $R_f$ (9:1 *v/v* $CH_2Cl_2/CH_3OH$) = 0.45; $^1$H NMR (300 MHz, [D$_6$]acetone): $\delta$ = 7.52–7.49 (2H, m, 2×Ar-H), 7.38 (2H, d, $J$ = 8.7 Hz, 2×Ar-H), 7.30 (2H, d, $J$ = 8.7 Hz, 2×Ar-H), 6.92 (1H, d, $J$ = 8.8 Hz, Ar-H), 4.45 ppm (2H, s, $CH_2$); $^{13}$C NMR (75 MHz, [D$_6$]acetone): $\delta$ = 193.1, 151.9, 146.3, 136.5, 132.8, 131.9, 130.1, 129.5, 123.8, 116.6, 116.1, 41.2 ppm; IR (ATR): $\tilde{\nu}$ = 3545 (br. OH str.), 3442 (br. OH str.), 1709 cm$^{-1}$; HRMS calcd for $C_{14}H_{11}ClO_3SNa$ $[M+Na]^+$ 317.0010, found 317.0015.

**1-(3,4-Dihydroxyphenyl)-2-(naphthalen-2-ylthio)ethanone (16n)**: Acetonide-based inhibitor **15n** (30 mg, 0.086 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (98:2 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16n** as a white solid (20 mg, 75%). $R_f$ (9:1 *v/v* $CH_2Cl_2/CH_3OH$) = 0.71; mp: 125–126 °C (dec.); $^1$H NMR (400 MHz, [D$_6$]acetone): $\delta$ = 7.95–7.84 (4H, m, 4×Ar-H), 7.63–7.60 (2H, m, 2×Ar-H), 7.57–7.48 (3H, m, 3×Ar-H), 6.98 (1H, d, $J$ = 8.0 Hz, Ar-H), 4.62 ppm (2H, s, $CH_2$); $^{13}$C NMR (100 MHz, [D$_6$]acetone): $\delta$ = 192.1, 150.7, 145.1, 134.0, 133.9, 131.9, 128.4, 128.3, 127.7, 127.1, 127.0, 126.7, 126.5, 125.8, 122.6, 115.3, 114.9, 39.8 ppm; IR (ATR): $\tilde{\nu}$ = 3470 (br. OH str.), 3384 (br. OH str.), 1667 cm$^{-1}$; HRMS calcd for $C_{18}H_{14}O_3SNa$ $[M+Na]^+$ 333.0556, found 333.0554.

**2-(Benzo[*d*]thiazol-2-ylthio)-1-(3,4-dihydroxyphenyl)ethanone (16o)**: Acetonide-based inhibitor **15o** (6 mg, 0.017 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (98:2 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16o** as a yellow oil (4.4 mg, 83%). $R_f$ (9:1 *v/v* $CH_2Cl_2/CH_3OH$) = 0.37; $^1$H NMR (400 MHz, [D$_6$]acetone): $\delta$ = 7.95 (1H, d, $J$ = 8.0 Hz, Ar-H), 7.81 (1H, d, $J$ = 8.0 Hz, Ar-H), 7.63 (1H, dd, $J$ = 1.8, 8.0 Hz, Ar-H), 7.60 (1H, d, $J$ = 1.8 Hz, Ar-H), 7.46 (1H, app.t, $J$ = 8.0 Hz, Ar-H), 7.36 (1H, app.t, $J$ = 8.0 Hz, Ar-H), 6.99 (1H, d, $J$ = 8.0 Hz, Ar-H), 5.03 ppm (2H, s, $CH_2$); $^{13}$C NMR (100 MHz, [D$_6$]acetone): $\delta$ = 190.5, 167.0, 153.2, 151.0, 145.2, 135.3, 128.2, 126.2, 124.4, 122.4, 121.4, 121.3, 115.2, 115.0, 40.3 ppm; IR (ATR): $\tilde{\nu}$ = 3287 (br. OH str.), 1671 cm$^{-1}$; MS (ESI): *m/z* 318 $[M+H]^+$.

**1-(3,4-Dihydroxyphenyl)-2-((4,5-diphenyloxazol-2-yl)thio)ethanone (16p)**: Acetonide-based inhibitor **15p** (40 mg, 0.090 mmol) was deprotected using the general procedure described above at room temperature for 1.5 h and purified by column chromatography (9:1 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16p** as a white solid (30 mg, 83%). $R_f$ (95:5 *v/v* $CH_2Cl_2/CH_3OH$) = 0.35; mp: 190–191 °C (dec.); $^1$H NMR (400 MHz, [D$_6$]DMSO): $\delta$ = 7.51–7.38 (12H, m, 12× Ar-H), 6.85 (1H, d, $J$ = 8.5 Hz, Ar-H), 4.96 ppm (2H, s, $CH_2$); $^{13}$C NMR (100 MHz, [D$_6$]DMSO): $\delta$ = 191.4, 158.8, 151.5, 146.7, 145.8, 136.2, 132.1, 129.4, 129.2, 127.9, 126.6, 122.6, 115.7, 115.6, 40.2 ppm; IR (ATR): $\tilde{\nu}$ = 3257 (br. OH str.), 1659 cm$^{-1}$; HRMS calcd for $C_{23}H_{18}NO_4S$ $[M+H]^+$ 404.0951, found 404.0951.

**2-((2-(3,4-Dihydroxyphenyl)-2-oxoethyl)thio)quinazolin-4(3*H*)-one (16q)**: Acetonide-based inhibitor **15q** (18 mg, 0.049 mmol) was deprotected using the general procedure described above at 50 °C for 5 h and purified by column chromatography (9:1 *v/v* $CH_2Cl_2/CH_3OH$) to afford inhibitor **16q** as a white solid (16 mg, 83%). $R_f$ (9:1 *v/v* $CH_2Cl_2/CH_3OH$) = 0.48; mp: 214–215 °C; $^1$H NMR (400 MHz, [D$_6$]DMSO): $\delta$ = 9.99 (1H, s, OH), 9.43 (1H, s, OH), 8.01 (1H, d, $J$ = 8.2 Hz, Ar-H), 7.70 (1H, dt, $J$ = 1.1, 8.0 Hz, Ar-H), 7.53 (1H, dd, $J$ = 1.7, 8.5 Hz, Ar-H), 7.43 (1H, d, $J$ = 1.7 Hz, Ar-H), 7.39 (1H, app.t, $J$ = 8.0 Hz, Ar-H), 7.27 (1H, app.d, $J$ = 8.2 Hz, Ar-H), 6.88 (1H, d, $J$ = 8.2 Hz, Ar-H), 4.76 ppm (2H, s, $CH_2$); $^{13}$C NMR (100 MHz, [D$_6$]DMSO): $\delta$ = 191.7, 161.5, 155.5, 151.5, 148.7, 145.6, 135.1, 128.2, 126.5, 126.2, 126.1, 122.3, 120.3, 115.6 (×2), 37.5 ppm; IR (ATR): $\tilde{\nu}$ = 3164 (br. OH str.), 1667 cm$^{-1}$; HRMS calcd for $C_{16}H_{13}N_2O_4S$ $[M+H]^+$ 329.0591, found 329.0590.

### *Biological assays*

***M. tuberculosis* type II DHQase assay**: Enzyme assays for type II DHQase from *M. tuberculosis* (overexpressed and purified as described previously)[15,23,24] were carried out using a Shimadzu UV/Vis 1800 spectrometer with a 6×6 Peltier cell holder using 1 cm path length quartz cuvettes at $\lambda$ 234 nm to monitor the formation of the product, 3-dehydroshikimate. Initial reaction rates were measured by the increase in absorbance at 234 nm from formation of the enone–carboxylate chromophore of 3-dehydroshikimate ($\varepsilon$ = $1.2\times10^4$ M$^{-1}$cm$^{-1}$). The assays were performed at 25 °C in Tris·HCl buffer (0.05 M, pH 7.0) for *M. tuberculosis* type II DHQase. The assays contained 3.80 nM enzyme, and were performed in duplicate. The assay mixtures were prepared in 1 mL quartz cuvettes, and the assays were initiated by the addition of the substrate (3-dehydroquinate, synthesized by a published procedure)[26] to the mixture after incubating the buffer, inhibitor in 0.5% DMSO, and enzyme at 25 °C for 3 min. The kinetic data for inhibition studies were obtained by measuring the initial rates of reaction over a range of inhibitor concentrations (3–4 different concentrations) at five different substrate concentrations (0.9$K_M$–4$K_M$). The inhibition constants ($K_i$) and the standard deviations were determined using nonlinear regression fitting to the competitive model by GraphPad Prism (version 5.03 for Windows). Kinetic data that were poorly fitted to a competitive model ($R^2$ < 0.95) were fitted to a mixed inhibition model in GraphPad Prism using nonlinear regression to derive the inhibition constants ($K_i$) and the standard deviations.

### *Microplate-based assay with Alamar blue (resazurin) readout for* M. tuberculosis

*M. tuberculosis* H37Ra (ATCC 25177) was grown in Middlebrook 7H9 broth medium supplemented with OADC (Difco Laboratories, Detroit, MI, USA), 0.5% glycerol, and 0.05% Tween-80. Freshly seeded cultures were grown at 37 °C, for approximately 14 days, to mid-exponential phase ($OD_{600}$ 0.4–0.8) for use in the inhibition assays. The effect of the type II DHQase inhibitors against *M. tuberculosis* growth were measured by a resazurin reduction microplate assay, using the procedure previously described by Taneja and Tyagi.[23a] *M. tuberculosis* grown to mid-exponential phase ($OD_{600}$ 0.4–0.8) was diluted to $OD_{600}$ 0.002 in 7H9S media (Middlebrook 7H9 with OADC, 0.5% glycerol, 0.02% tyloxapol, 1% tryptone) containing 0.5% DMSO; 96-well microtiter plates were set up with 100 μL inhibitors, serially diluted into 7H9S. Diluted *M. tuberculosis* (100 μL, representing ~$2\times10^4$ CFU mL$^{-1}$) was added to each well. Plates were incubated for 5 days at 37 °C in a humidified incubator prior to the addition of a 0.02% resazurin solution (30 μL) and 20% Tween-80 (12.5 μL) to each well. Sample fluorescence was measured after 48 h on a BMG Labtech Polarstar Omega in-

strument with an excitation wavelength of 530 nm and emission at 590 nm. Changes in fluorescence relative to positive control wells (H37Ra with no inhibitor) minus negative control wells (no H37Ra) were plotted for determination of $MIC_{50}$ values.

## *Acknowledgements*

*We thank the Australian National Health and Medical Research Council for funding (Project Grant number 632769) and PhD support from a John Lamberton Research Scholarship and an Australian Postgraduate Award (ATT). We also thank Dr. Adrian Lapthorn (Department of Chemistry and Division of Biochemistry and Life Science, University of Glasgow) for providing the plasmid for* M. tuberculosis *type II DHQase and Ms. Angel Pang for technical assistance.*

**Keywords:** antibiotics · dehydroquinases · inhibitors · medicinal chemistry · *Mycobacterium tuberculosis*

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Received: December 23, 2011
Revised: March 7, 2012
Published online on ■■ ■■, 0000

  

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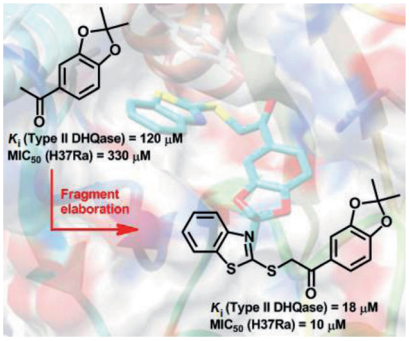
**Elucidation of *Mycobacterium tuberculosis* Type II Dehydroquinase Inhibitors using a Fragment Elaboration Strategy**




**Extend to improve**: Novel inhibitors of *Mycobacterium tuberculosis* type II dehydroquinase were discovered through a fragment elaboration approach. A number of low-micromolar inhibitors of the enzyme were elucidated which possess significant activity against *M. tuberculosis* in vitro.