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PAPER

Synthesis and evaluation of *M. tuberculosis* salicylate synthase (MbtI) inhibitors designed to probe plasticity in the active site†

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Mycobacterium tuberculosis salicylate synthase (MbtI) catalyses the first committed step in the biosynthesis of mycobactin T, an iron-chelating siderophore essential for the virulence and survival of *M. tuberculosis*. Co-crystal structures of MbtI with members of a first generation inhibitor library revealed large inhibitor-induced rearrangements within the active site of the enzyme. This plasticity of the MbtI active site was probed *via* the preparation of a library of inhibitors based on a 2,3-dihydroxybenzoate scaffold with a range of substituted phenylacrylate side chains appended to the C3 position. Most compounds exhibited moderate inhibitory activity against the enzyme, with inhibition constants in the micromolar range, while several dimethyl ester variants possessed promising anti-tubercular activity *in vitro*.

Introduction

Tuberculosis (TB), caused by infection with the bacterium *Mycobacterium tuberculosis*, is responsible for significant morbidity and mortality worldwide. In 2010, TB caused 1.5 million deaths and a further 8.8 million new *M. tuberculosis* infections were identified.[1] The emergence of multi-drug and extensively-drug resistant strains of *M. tuberculosis*, resistant to first or first and second line therapies, respectively, have now been reported on every major continent.[2] There is thus an urgent need for the development of anti-tubercular agents with novel modes of action.

M. tuberculosis salicylate synthase (SS), or MbtI, belongs to a family of chorismate-utilising enzymes (Scheme 1).[3] Enzymes of this class, present in bacteria, fungi, plants and apicomplexan parasites, are responsible for the conversion of chorismate (**1**) into a range of essential aromatic building blocks. For example anthranilate synthase (AS), 4-amino-4-deoxychorismate (ADC) synthase and isochorismate synthase (IS) catalyse the first steps in the biosynthesis of tryptophan, folate and siderophores, respectively. Given the importance of these secondary metabolites, there has been significant attention directed toward the development of inhibitors of these enzymes.[4–10]

MbtI, like other salicylate synthases, is responsible for the conversion of chorismate (**1**) into salicylate (**2**) through an enzyme bound intermediate, isochorismate (**3**) (Scheme 2).[3,11] In *M. tuberculosis*, generation of salicylate is the first committed step towards the biosynthesis of the siderophores mycobactin T (**4**) and carboxymycobactin T (not shown), responsible for sequestration of iron from the host.[12–15] Due to the essential nature of iron for the growth and continued survival of *M. tuberculosis*, MbtI represents an appealing target for the development of new TB drug candidates.

We recently reported the first inhibitors of MbtI (*e.g.* **5** and **6** in Fig. 1).[16] These compounds, based on a 2,3-dihydroxybenzoate scaffold, to mimic the intermediate isochorismate (**3**), exhibited low micromolar inhibition of the enzyme (K_i = 11–21 μM). More recently, Aldrich and co-workers have reported a different class of non-competitive benzimidathiazole-based MbtI inhibitors (IC_{50} = 7–9 μM) based on a high throughput screen of >100 000 commercial compounds.[17]

Results and discussion

Prior inhibitors synthesised in our laboratory (including **5** and **6**) were recently screened against *M. tuberculosis* (H37Ra strain) *in vitro*. Unfortunately, all compounds exhibited poor antibacterial activity (MIC_{50} > 1 mM). We hypothesised that the lack of anti-

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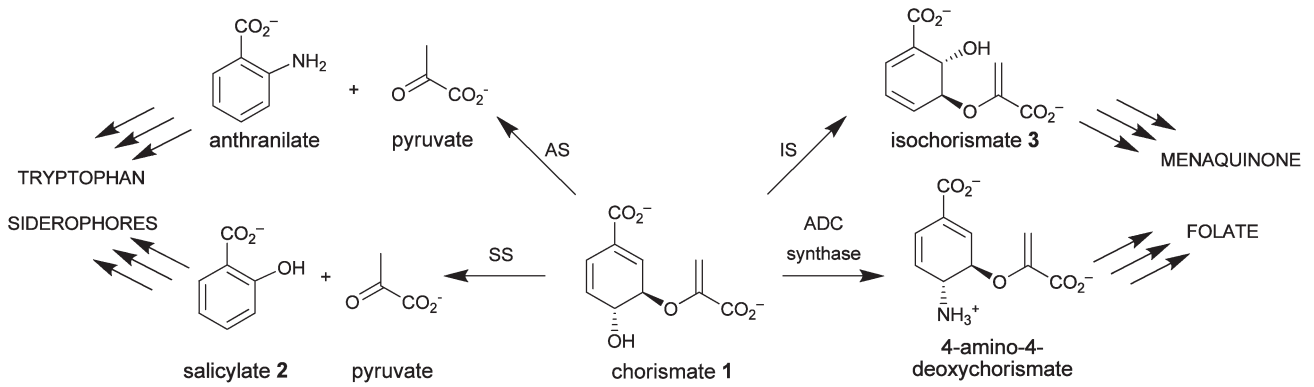
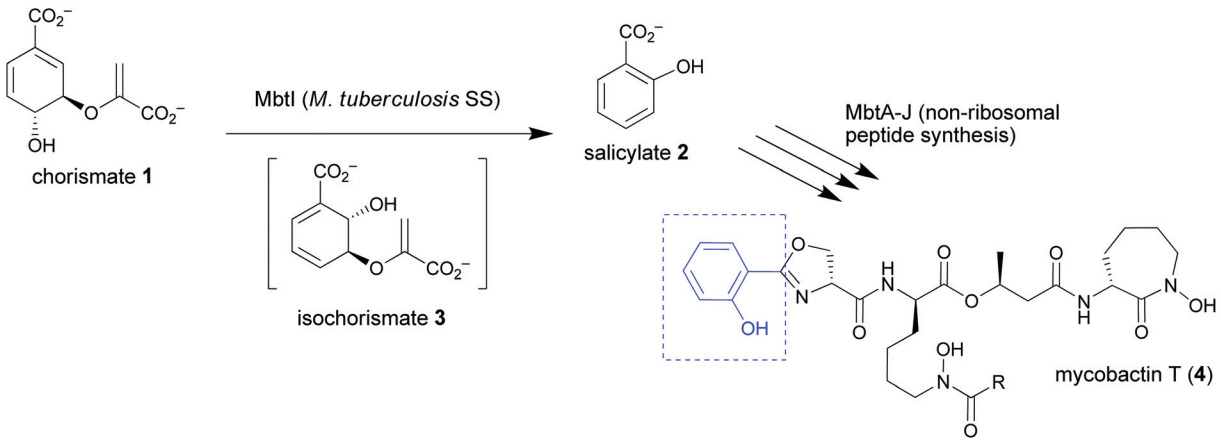
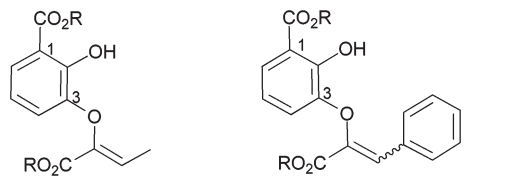
†Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of all novel compounds and molecular dockings of **25–38**. See DOI: 10.1039/c2ob26736e



Scheme 1 Transformations achieved by the chorismate-utilising enzymes: salicylate synthase (SS), anthranilate synthase (AS), isochorismate synthase (IS) and 4-amino-4-deoxychorismate (ADC) synthase.



Scheme 2 Activity of MbtI, the salicylate synthase (SS) of *Mycobacterium tuberculosis*, showing the enzyme bound intermediate, isochorismate **3**, and siderophore product, mycobactin T (**4**).



5: R=H; K_i (MbtI) = 11 ± 1 μM
6: R=H; K_i (MbtI) = 21 ± 5 μM
7: R=Me; MIC_{50} = 792 ± 22 μM
8: R=Me; MIC_{50} = 277 ± 19 μM

Fig. 1 Previously reported inhibitors of *M. tuberculosis* salicylate synthase with K_i values against MbtI and MIC_{50} values against *M. tuberculosis*.[16] NB: **7** and **8** are prodrugs of **5** and **6** and were screened against *M. tuberculosis* in a whole cell assay.

tubercular activity was owing to the highly hydrophilic nature of the molecules together with the negative charge from the dicarboxylate moieties present in these compounds, thus making it difficult for these to efficiently penetrate the waxy mycobacterial cell wall.[18] We therefore evaluated methyl ester variants of these compounds in the whole cell assays against the bacterium (designed as prodrugs). This led to a significant enhancement in potency, compared with the free acids, with MIC_{50} values in the micromolar range (see Fig. 1 for MIC_{50} values of dimethyl esters **7** and **8**). These results provided impetus for the design and synthesis of a new series of inhibitors that target MbtI and perhaps other chorismate-utilising enzymes operating in *M. tuberculosis* which possess antibacterial activity.

Inhibitor design

We recently obtained co-crystal structures of MbtI with inhibitors **5** and **6**, bearing 3-methylacrylate- and 3-phenylacrylate side chains bound in the active site.[19] These structures have provided new insights into the binding mode of these compounds, notably through the observation of large inhibitor-induced rearrangements of the protein structure around the active site. It is important to note that this flexibility of active site residues was not taken into account in previous rigid-receptor docking predictions used for guiding prior inhibitor design and, as such, led to a predicted binding mode (from prior docking studies) which was significantly different to that observed in the co-crystal structure (see Fig. 2 for comparison).[16]

For example, the inhibitor-bound MbtI crystal structures clearly showed the C3 side chain carboxylate oriented towards the metal binding site, whilst the C1 carboxylate is positioned between Arg405 and Gly419 to form favourable ionic and hydrogen bonding interactions with these residues, respectively

(Fig. 3a–c).[19] Relative to the MbtI structure used for docking studies, there were changes in the sidechain positions for residues Glu252, Thr361 and Arg405 that helped accommodate the extended enol sidechain of **5** in this binding mode. Binding of **6** (bearing the bulky 3-phenylacrylate side chain) as the *E* or *Z* isomer required much more extensive rearrangements of the MbtI structure. This was achieved by significant movement of the peptide backbone in regions adjacent to the phenyl substituent (Fig. 3).[19] Specifically, residues 268–270 (within a strand of a β-sheet) were significantly displaced by the larger side chain containing compounds, with the greatest displacement observed in the 3-phenylacrylate-based inhibitors (Fig. 3a–c). The co-crystal structures of MbtI with inhibitor **6** bound showed that both *E* and *Z* isomers could be accommodated in the active site cleft (Fig. 3b and c).[19] The side chains of these two diastereoisomers occupied very different regions of the active site. The most striking observation was that a three-stranded β-sheet that lines the enol-pyruvyl binding pocket was displaced from the active site to accommodate the phenyl side chains of both the *E*- and the *Z*-isomers. The local effect of this rearrangement was the opening of a cavity in a region previously occupied by the

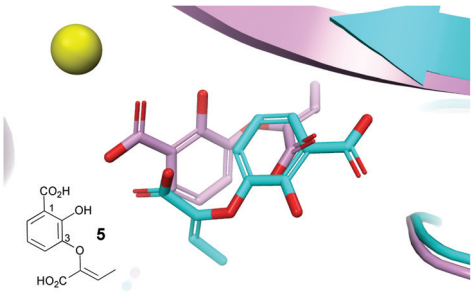


Fig. 2 Predicted binding mode of inhibitor **5** in the active site of MbtI (pink) overlaid with the binding mode observed in the co-crystal structure (blue, PDB ID: 3VEH). NB: The Mg^{2+} ion (yellow) was not present in the crystal structures and was modelled into the active site.

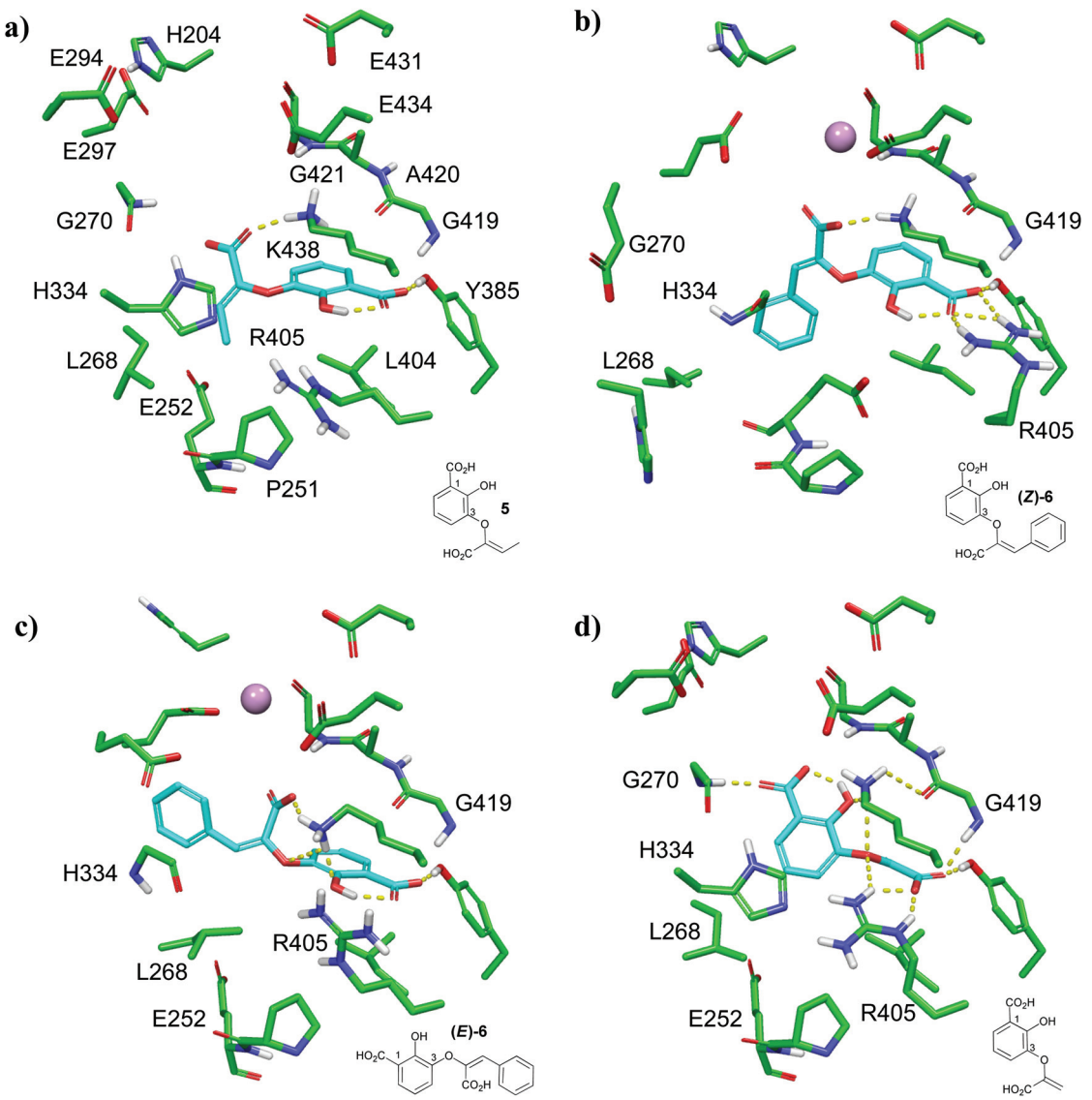


Fig. 3 Co-crystal structure of MbtI with (a) inhibitor **5** bound, (b) inhibitor (*Z*)-**6** bound, (c) inhibitor (*E*)-**6** bound and (d) aromatic inhibitor bearing native enol-pyruvyl side chain bound.[19] PDB ID: (a) 3VEH, both (b) and (c) 3RV6, (d) 3ST6.

β-strand. The *Z*-isomer further induces movement of Glu252 and Thr361 again facilitated by changes in the position of the peptide backbone. The *Z*-isomer also revealed a further alternative positioning for Arg405, in which it twists around to form a direct salt-bridge to the C1 carboxylate. This interaction is not observed in the *E*-isomer or the crystal structure with **5** (bearing a 3-methylacrylate side chain) bound. Taken together, these observations indicate significant plasticity in the active site of MbtI.

Interestingly, the binding mode (and movement of active site residues) seen for inhibitors **5** and **6** is not observed when the native enol-pyruvyl side chain (present in chorismate and isochorismate) is incorporated into an inhibitor (see Fig. 3d for co-crystal structure). In this case the inhibitor binds in the same mode predicted by our previous docking studies (with the C1 carboxylate interacting with the Mg^{2+} ion and the C3 side chain carboxylate forming interactions with the side chains of Arg405 and Tyr305). This compound is over an order of magnitude less potent as an inhibitor of MbtI compared to **5** and **6** (with extended side chains) suggesting that there are significantly improved interactions with active site residues when compounds bind in the alternate mode observed for compounds **5** and **6** possessing extended enol side chains at C3.

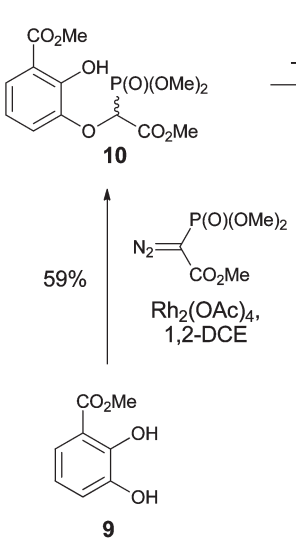
Given these observations we were interested in exploiting the plasticity of the active site and the inducible cavities by designing inhibitors bearing a range of *meta*-, *ortho*- or *para*-substituents around the aryl ring of the 3-phenylacrylate side chain of inhibitor **6**. This would enable a more detailed understanding of which functional groups could be incorporated to facilitate additional interactions with the induced pocket and hopefully provide inhibitors with increased potency against the enzyme. Use of substituted aromatic side chains in the proposed library was further justified by the resulting increase in sidechain lipophilicity, which was expected to increase anti-tubercular activity as has been reported previously.[20]

The proposed inhibitor library was docked into the active site of the co-crystal structure of MbtI with (*Z*)-**6** and (*E*)-**6** bound (PDB ID: 3RV6) using Glide® (Schrödinger).[21] Bromo-, chloro-, hydroxyl-, methyl- or trifluoromethyl substituents on the aryl ring of the C3 side chain were chosen to investigate the effect of *ortho*-, *meta*- and *para*-substitution (see ESI†). In the case of the *para*-substituted trifluoromethylphenylacrylate side chain, *in silico* studies suggested a number of unfavourable steric interactions and therefore this compound was removed from the final target library. The remainder of the inhibitors (14 in total in both *E*- and *Z*-diastereomeric configurations) showed favourable binding modes in the active site as gauged by visual inspection and docking scores (see ESI†). We therefore chose to embark on the synthesis of these compounds.

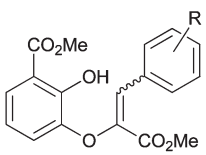
Synthesis

Synthesis of the inhibitors began from key phosphonate **10**.[16] This was obtained *via* a rhodium-carbenoid insertion between diazophosphonoacetate and methyl 2,3-dihydroxybenzoate **9** (Scheme 3). The key phosphonate **10** was then reacted with a range of substituted benzaldehydes *via* a Horner–Wadsworth–Emmons (HWE) reaction. Specifically, phosphonate **10** was deprotonated with lithium hexamethyldisilazide (LiHMDS) at −78 °C and reacted with the range of aldehydes to give the desired dimethyl esters **11**–**24** in good to excellent yields (50–87%). It is important to note that **11**–**24** were obtained as mixtures of *Z*- and *E*-diastereoisomers and, in most cases, could not be easily separated *via* column chromatography. Nonetheless, it was possible in some cases to isolate fractions of either pure *Z* (compounds **12**, **13**, **15**, and **24**) or *Z*-rich fractions, however, *E*-rich fractions could not be obtained.

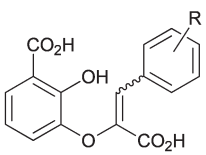
Saponification of methyl esters **11**–**24** with aqueous potassium hydroxide followed by acidification with 1 M hydrochloric acid



LiHMDS, R-PhCHO, THF, -78 °C



i) 2M KOH, THF:H_2O
ii) HCl workup



11 R = *m*-Br (84%, 50:50 *Z*:*E*)
12 R = *o*-Br (78%, 86:14 *Z*:*E*)
13 R = *p*-Br (64%, 70:30 *Z*:*E*)
14 R = *m*-Cl (87%, 56:44 *Z*:*E*)
15 R = *o*-Cl (57%, 91:9 *Z*:*E*)
16 R = *p*-Cl (70%, 67:33 *Z*:*E*)
17 R = *m*-Me (82%, 56:44 *Z*:*E*)
18 R = *o*-Me (60%, 53:47 *Z*:*E*)
19 R = *p*-Me (50%, 67:33 *Z*:*E*)
20 R = *m*-OAc (56%, 41:59 *Z*:*E*)
21 R = *o*-OAc (60%, 67:33 *Z*:*E*)
22 R = *p*-OAc (61%, 56:44 *Z*:*E*)
23 R = *m*-CF_3 (67%, 59:41 *Z*:*E*)
24 R = *o*-CF_3 (67%, 91:9 *Z*:*E*)

25 R = *m*-Br (54%, 63:37 *Z*:*E*)
26 R = *o*-Br (quant, *Z* only)
27 R = *p*-Br (79%, *Z* only)
28 R = *m*-Cl (86%, 59:41 *Z*:*E*)
29 R = *o*-Cl (quant, *Z* only)
30 R = *p*-Cl (86%, 83:17 *Z*:*E*)
31 R = *m*-Me (76%, 63:37 *Z*:*E*)
32 R = *o*-Me (82%, 56:44 *Z*:*E*)
33 R = *p*-Me (61%, 67:33 *Z*:*E*)
34 R = *m*-OH (56%, 63:37 *Z*:*E*)
35 R = *o*-OH (38%, *Z* only)
36 R = *p*-OH (59%, *Z* only)
37 R = *m*-CF_3 (80%, 77:23 *Z*:*E*)
38 R = *o*-CF_3 (75%, *Z* only)

Scheme 3 Synthesis of inhibitors **25**–**38**.

gave the desired inhibitors **25–38** in 38–86% yield (Scheme 3). It should be noted that in some cases the $Z:E$ isomer ratios observed do not directly reflect the ratios of the corresponding di-methyl esters used in the preceding saponification reactions. This change in diastereomeric ratio was owing to acid-catalysed olefin isomerisation during work-up (see diastereomeric ratios in parentheses).

MbtI inhibition studies

With compounds **25–38** in hand, these were next screened against MbtI using a lactate dehydrogenase coupled assay as reported previously.[16] All compounds exhibited competitive, reversible inhibition of MbtI with inhibition constants (K_i) ranging from 45–186 μM (see Table 1). As such, all compounds possessed a reduced inhibitory potency when compared to unsubstituted inhibitor **6** and also exhibited a relatively flat structure–activity profile against the enzyme.

These results suggest that although the aryl moiety of the sidechain is accommodated into the active site *via* local movement of the backbone and side chain moieties of the enzyme, additional interactions with active site residues do not appear to be facilitated through substitution. Substitution at the *ortho*-position appeared to be better tolerated in the active site of MbtI than *para*- or *meta*-substitution leading to slightly better inhibition of the enzyme. This is highlighted by the halogen-substituted inhibitors (**25–30**) where compounds bearing *ortho*-Cl (**29**) and *ortho*-Br (**26**) substituted aryl sidechains possessed a two fold increase in activity when compared with the *meta*- and *para*-substituted compounds (**25**, **27**, **28** and **30**).

Inhibitors possessing methyl and trifluoromethyl substituted aryl side chains (Table 1: **31–33** and **37–38**) were consistently less potent inhibitors (albeit by a small difference) of the enzyme with K_i values between 110–186 μM.

Interestingly, the hydroxyl-substituted compounds **34–36** possessed almost identical inhibition of the enzyme irrespective of substitution around the aryl ring of the phenylacrylate side chain. The most potent of the inhibitors, the *o*-Cl and *o*-Br substituted compounds (**29** and **26**) may be indicative of the size, electronics and positioning of the substituent which is best tolerated in the ligand-induced pocket.

Inhibition of *M. tuberculosis* growth *in vitro*

Having established the inhibition of MbtI by inhibitors **25–38**, we were next interested in assessing the activity of these compounds against *M. tuberculosis* growth *in vitro*. This was carried out using a resazurin reduction microplate assay, previously described.[22–24] As our prior assays with the dicarboxylate inhibitors showed no activity against *M. tuberculosis*, thought to be due to inefficient permeability of the mycobacterial cell wall, the di-methyl ester precursors (**11–24**) were screened against the bacterium (Table 2).[18]

The vast majority of these compounds were significantly more potent than all previously screened MbtI inhibitors against *M. tuberculosis* with MIC_{50} values ranging from 25–460 μM (Table 2). Despite the increased potency against *M. tuberculosis in vitro*, there appeared to be no significant correlation between the potency against MbtI and the antibacterial activity of the corresponding di-methyl ester. This discrepancy may well be the result of more than one enzymatic target of *M. tuberculosis* being inhibited. This is most striking in the example of the *o*-CF_3 compound **38** which was a moderate inhibitor of MbtI (K_i = 125 μM) but was found to be the most potent inhibitor of bacterial growth with an MIC_{50} of 25 μM (for di-methyl ester **24**).

It is also feasible that these compounds exert their antibacterial activity *via* the inhibition of more than one chorismate-utilising enzyme in *M. tuberculosis*.[4–10] Given the range of enzymes that are known to convert chorismate to their respective products *via*

Table 1 Inhibition of MbtI by 3-phenylacrylate-based inhibitors **25–38**[a]

Compound	K_i MbtI (μM)	Compound	K_i MbtI (μM)	Compound	K_i MbtI (μM)
25 (*m*-Br)	86 ± 7.8	**26** (*o*-Br)	51 ± 3.7	**27** (*p*-Br)	70 ± 7.0
28 (*m*-Cl)	71 ± 5.5	**29** (*o*-Cl)	45 ± 4.3	**30** (*p*-Cl)	78 ± 6.6
31 (*m*-Me)	186 ± 14	**32** (*o*-Me)	110 ± 8.8	**33** (*p*-Me)	170 ± 20
34 (*m*-OH)	97 ± 7.9	**35** (*o*-OH)	92 ± 9.7	**36** (*p*-OH)	92 ± 13
37 (*m*-CF_3)	153 ± 11	**38** (*o*-CF_3)	125 ± 13	**6** (Ph)	21 ± 5

[a] Kinetic constants: MbtI K_M = 2.2 ± 0.2 μm, k_{cat} = 0.8 ± 0.1 min^{-1}.

Table 2 Inhibition of *M. tuberculosis* by 3-phenylacrylate-based methyl ester prodrugs **11–24**[a]

Compound	MIC_{50} (μM)	Compound	MIC_{50} (μM)	Compound	MIC_{50} (μM)
11 (*m*-Br)	248 ± 26	**12** (*o*-Br)	95 ± 29	**13** (*p*-Br)	188 ± 14
14 (*m*-Cl)	204 ± 18	**15** (*o*-Cl)	118 ± 32	**16** (*p*-Cl)	203 ± 24
17 (*m*-Me)	263 ± 51	**18** (*o*-Me)	140 ± 47	**19** (*p*-Me)	270 ± 33
20 (*m*-OH)	460 ± 36	**21** (*o*-OAc)	89 ± 15	**22** (*p*-OAc)	120 ± 18
23 (*m*-CF_3)	180 ± 19	**24** (*o*-CF_3)	25 ± 5	**8** (Ph)	277 ± 19

[a] Compounds were tested in an Alamar blue (Resazurin) assay, rifampicin MIC_{50} = 1.3 ± 0.4 nM, isoniazid MIC_{50} = 198 ± 4.4 nM. Data represent mean ± SD from three replicate assays for *M. tuberculosis*.

similar mechanistic pathways it is possible that these are inhibited by the compounds synthesised here.[8,25] This theory may be further supported by the recent characterisation of a 4-amino-4-deoxychorismate lyase and an anthranilate synthase in *M. tuberculosis*.[26,27] Furthermore, previously investigated lipophilic inhibitors have exhibited greater potency against an anthranilate synthase (from *Serratia marcescens*) compared with MbtI.[16] Work is ongoing to investigate if inhibition of multiple biosynthetic pathways is occurring.

It should be noted that we conducted an additional screen of a selection of compounds against the H37Ra strain of *M. tuberculosis* under iron limiting conditions, where MbtI is known to be highly over-expressed (see ESI†). Under these conditions each of the inhibitors were significantly less effective at inhibiting the growth of the bacillus. MbtI is expected to be essential for viability under these iron limiting conditions. One interpretation of these results is that they stem from the over-expression of MbtI under iron limitation which would therefore require more compound for inhibition of growth. A more detailed investigation of these effects will be the subject of future work in our laboratories.

Conclusions

Recent co-crystal structures of MbtI were used to guide the design of a second generation library of novel phenylacrylate-derived inhibitors bearing a range of substituted aromatic side chains in order to probe for additional interactions within an inducible pocket. These compounds proved to be slightly less potent against MbtI (inhibition constants in the mid-micromolar range) compared with previous inhibitors synthesised in our laboratory suggesting that, although these large groups could be accommodated by movement of active site residues, no further interactions were made. Dimethyl ester variants of these compounds displayed significantly improved activity against the growth of *M. tuberculosis in vitro*. The most potent compound in this series was *o*-CF_3-derived compound **24** which exhibited an MIC_{50} of 25 μM against *M. tuberculosis* and will serve as a lead for the development of analogues. Future work in our laboratories will aim to assess the inhibition of other chorismate-utilising enzymes present in *M. tuberculosis* by these and other compounds.

Experimental

General synthesis procedures

NMR spectra were recorded at 300 K using Bruker Avance DRX200, DRX300, DPX400, or 500 spectrometers at a frequency of 200.1, 300.2, 400.2 or 500.2 MHz, respectively. ^{1}H NMR chemical shifts are reported in parts per million (ppm) and are referenced to solvent residual signals: $CDCl_3$ (δ = 7.26 ppm), MeOD (δ = 3.31 ppm) or D_2O (δ = 4.79 ppm). ^{1}H NMR data are reported as chemical shift (δ_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 **25–38** were analysed using a Waters Sunfire 5 μm, 2.1 × 150 mm column (C18) 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. 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 F254). 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 λ 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.

Procedure A: Horner–Wadsworth–Emmons reaction

Lithium bis(trimethylsilyl)amide (2 equiv. 1.0 M soln. in THF) was added dropwise over 5 min to a stirred solution of phosphonate **10** (1 equiv.) in THF (6 mL $mmol^{-1}$) at −78 °C. The mixture was stirred for a further 5 min before dropwise addition of aldehyde (2 equiv.). The mixture was stirred for 2–6 h at −78 °C before warming to rt. The reaction was quenched through the dropwise addition of saturated aqueous NH_4Cl solution (1–2 mL) followed by extraction of the aqueous layer with EtOAc (3 × 50 mL). The combined organic fractions were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The product was purified *via* column chromatography to afford diesters as mixtures of diastereomers.

Methyl 3-(1-(3-bromophenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 11. Phosphonate **10** (150 mg, 0.43 mmol) and *m*-bromobenzaldehyde (100 μL, 0.86 mmol) were reacted for 2 h at −78 °C according to Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 90 : 10 v/v) to afford diester **11** as a mixture of diastereomers as a yellow oil (147 mg, 84%, 50 : 50 *Z*/*E*). R_f [hexane : EtOAc 5 : 1 v/v] = 0.29; IR ν_{max} (ATR): 3118 (br), 2953, 1729, 1677, 1438, 1248 cm^{-1}; *Z*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.05 (1H, s, OH), 7.86 (1H, s, C=C*H*Ph), 7.71 (1H, app. d, J 7.9 Hz, Ar-H), 7.55 (1H, dd, J 1.2, 8.0 Hz, H-6), 7.45–7.38 (1H, m, Ar-H), 7.27–7.17 (2H, m, Ar-H), 6.96 (1H, app. d, J 8.0 Hz, H-4), 6.74 (1H, t, J 8.0 Hz, H-5), 3.96 (3H, s, CO_2Me), 3.76 (3H, s,

CO_2Me); *E*: ^1H NMR (400 MHz, $CDCl_3$) δ 11.10 (1H, s, OH), 7.66 (1H, dd, *J* 1.4, 8.1 Hz, Ar-H), 7.45–7.38 (2H, m, Ar-H + H-6), 7.27–7.17 (3H, m, Ar-H + H-4), 6.87 (1H, t, *J* 8.0 Hz, H-5), 6.38 (1H, s, C═C*H*Ph), 3.96 (3H, s, CO_2Me), 3.73 (3H, s, CO_2Me); (*E* + *Z* mix) ^{13}C NMR (100 MHz, $CDCl_3$) ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 (C═O), 170.3 (C═O), 163.3 (C═O), 162.9 (C═O), 152.9 (C), 151.8 (C), 144.6 (C), 144.4 (C), 143.3 (C), 141.1 (C), 135.3 (C), 134.3 (C), 133.0 (C), 132.4 (C), 131.6 (C), 130.6 (C), 130.1 (CH), 129.4 (CH), 128.6 (CH), 127.4 (CH), 125.6 (CH), 125.3 (CH), 125.0 (CH), 123.8 (CH), 122.6 (CH), 121.9 (CH), 120.2 (CH), 119.1 (CH), 118.7 (CH), 118.3 (CH), 114.0 (C), 113.6 (C), 52.6 (CH_3), 52.6 (CH_3), 52.5 (CH_3), 52.3 (CH_3); LRMS $[M + Na]^+$ 429.07; HRMS calcd for $C_{18}H_{15}O_6BrNa$: *M*Na^+, 428.9950. Found: *M*Na^+, 428.9940.

Methyl 3-(1-(2-bromophenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 12. Phosphonate **10** (150 mg, 0.44 mmol) and *o*-bromobenzaldehyde (160 mg, 0.87 mmol) were reacted for 2 h at −78 °C under Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 5 : 1 v/v) to afford diester **12** as a mixture of diastereomers as a white solid (140 mg, 78%, 86 : 14 *Z*/*E*). IR ν_{max} (ATR): 3106 (br), 2953, 1731, 1679, 1464, 1438, 1250 cm^{-1}; *Z*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.35; ^1H NMR (400 MHz, $CDCl_3$) δ 11.05 (1H, s, OH), 8.03 (1H, dd, *J* 1.4, 7.7 Hz, Ar-H), 7.70 (1H, s, C═C*H*Ph), 7.58 (1H, d, *J* 7.7 Hz, Ar-H), 7.52 (1H, dd, *J* 1.1, 8.0 Hz, H-6), 7.23 (1H, app. t, *J* 7.7 Hz, Ar-H), 7.13 (1H, dt, *J* 1.4, 7.7, 7.7 Hz, Ar-H), 6.98 (1H, dd, *J* 1.1, 8.0 Hz, H-4), 6.73 (1H, app. t, *J* 8.0 Hz, H-5), 3.95 (3H, s, CO_2Me), 3.78 (3H, s, CO_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (C═O), 163.5 (C═O), 152.0 (C), 145.0 (C), 141.4 (C), 133.0 (CH), 132.2 (C), 131.2 (CH), 130.7 (CH), 127.7 (CH), 125.2 (C), 124.8 (CH), 123.8 (CH), 120.5 (CH), 118.5 (CH), 113.7 (C) 52.8 (CH_3), 52.6 (CH_3); *E*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.27; ^1H NMR (400 MHz, $CDCl_3$) δ 11.04 (1H, s, OH), 7.67 (1H, dd, *J* 1.3, 8.0 Hz, H-6), 7.55 (1H, d, *J* 7.9 Hz, Ar-H), 7.32 (1H, app. t, *J* 7.7 Hz, Ar-H), 7.31–7.27 (2H, m, Ar-H + H-4), 7.16–7.11 (1H, m, Ar-H), 6.88 (1H, app. t, *J* 8.0 Hz, H-5), 6.51 (1H, s, C═C*H*Ph), 3.97 (3H, s, CO_2Me), 3.66 (3H, s, CO_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C═O), 163.5 (C═O), 153.1 (C), 144.4 (C), 143.8 (C), 134.6 (C), 130.8 (CH), 129.4 (CH), 126.9 (CH), 125.7 (CH), 125.3 (CH), 125.2 (C), 123.6 (C), 121.5 (CH), 118.9 (CH), 114.1 (C) 52.7 (CH_3), 52.4 (CH_3); LRMS $[M + Na]^+$ 430.87; HRMS calcd for $C_{18}H_{15}O_6BrNa$: *M*Na^+, 428.9950. Found: *M*Na^+, 428.9944.

Methyl 3-(1-(4-bromophenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 13. Phosphonate **10** (150 mg, 0.44 mmol) and *p*-bromobenzaldehyde (160 mg, 0.87 mmol) were reacted for 2 h at −78 °C under Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 5 : 1 v/v) to afford diester **13** as a mixture of diastereomers as a yellow oil (110 mg, 64%, 70 : 30 *Z*/*E*). IR ν_{max} (ATR): 3114 (br), 2953, 2923, 2851, 1729, 1679, 1464, 1439, 1249 cm^{-1}; *Z*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.42; ^1H NMR (400 MHz, $CDCl_3$) δ 11.08 (1H, s, OH), 7.61 (2H, d, *J* 8.6 Hz, Ar-H), 7.54 (1H, dd, *J* 1.4, 8.0 Hz, H-6), 7.46 (2H, d, *J* 8.6 Hz, Ar-H), 7.30 (1H, s, C═C*H*Ph), 6.95 (1H, dd, *J* 1.4, 8.0 Hz, H-4), 6.73 (1H, app. t, *J* 8.0 Hz, H-5), 3.97 (3H, s, CO_2Me), 3.75 (3H, s, CO_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (C═O), 163.7 (C═O), 152.0 (C), 144.8 (C), 140.7 (C), 132.1 (2 × CH), 131.9 (2 × CH), 131.4 (C), 125.9 (CH), 124.1 (C), 123.9 (CH), 120.2 (CH), 118.6 (CH), 113.8 (C), 52.7 (CH_3), 52.7 (CH_3); *E*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.36; ^1H NMR (400 MHz, $CDCl_3$) δ 11.01 (1H, s, OH), 7.66 (1H, dd, *J* 1.6, 8.2 Hz, H-6), 7.43 (2H, d, *J* 8.6 Hz, Ar-H), 7.24 (1H, dd, *J* 1.6, 8.2 Hz, H-4), 7.19 (2H, d, *J* 8.6 Hz, Ar-H), 6.86 (1H, app. t, *J* 8.2 Hz, H-5), 6.41 (1H, s, C═C*H*Ph), 3.97 (3H, s, CO_2Me), 3.72 (3H, s, CO_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C═O), 163.2 (C═O), 153.0 (C), 143.9 (C), 143.8 (C) 132.3 (C), 131.3 (2 × CH), 130.7 (2 × CH), 125.6 (CH), 125.2 (CH), 122.1 (C), 120.6 (CH), 118.9 (CH), 114.2 (C), 52.7 (CH_3), 52.5 (CH_3); LRMS $[M + Na]^+$ 430.87; HRMS calcd for $C_{18}H_{15}O_6BrNa$: *M*Na^+, 428.9950. Found: *M*Na^+, 428.9944.

Methyl 3-(1-(3-chlorophenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 14. Phosphonate **10** (150 mg, 0.43 mmol) and *m*-chlorobenzaldehyde (96 μL, 0.86 mmol) were reacted for 2 h at −78 °C according to Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 90 : 10 v/v) to afford diester **14** as a mixture of diastereomers as a yellow oil (126 mg, 87%, 56 : 44 *Z*/*E*). R_f [hexane : EtOAc 5 : 1 v/v] = 0.29; IR ν_{max} (ATR): 3115 (br), 2955, 1729, 1677, 1438, 1249 cm^{-1}; *Z*: ^1H NMR (400 MHz, $CDCl_3$) δ 11.04 (1H, s, OH), 7.71 (1H, s, C═C*H*Ph), 7.67–7.62 (1H, m, Ar-H), 7.28–7.15 (4H, m, Ar-H + H-6), 6.97 (1H, app. d, *J* 7.9 Hz, H-4), 6.73 (1H, t, *J* 7.9 Hz, H-5), 3.95 (3H, s, CO_2Me), 3.74 (3H, s, CO_2Me); *E*: ^1H NMR (400 MHz, $CDCl_3$) δ 11.08 (1H, s, OH), 7.67–7.62 (1H, m, Ar-H), 7.54 (1H, app. d, *J* 8.0 Hz, H-6), 7.28–7.15 (4H, m, Ar-H + H-4), 6.85 (1H, t, *J* 7.9 Hz, H-5), 6.38 (1H, s, C═C*H*Ph), 3.95 (3H, s, CO_2Me), 3.71 (3H, s, CO_2Me); (*E* + *Z* mix) ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 (C═O), 170.3 (C═O), 163.3 (C═O), 162.9 (C═O), 152.9 (C), 151.9 (C), 144.6 (C), 144.4 (C), 143.4 (C), 141.0 (C), 135.0 (C), 134.4 (C), 134.0 (C), 133.7 (C), 130.1 (C), 129.9 (C), 129.5 (CH), 129.2 (CH), 128.8 (CH), 128.3 (CH), 127.8 (CH), 127.0 (CH), 125.6 (CH), 125.3 (CH), 125.1 (CH), 123.8 (CH), 120.2 (CH), 119.3 (CH), 118.7 (CH), 118.3 (CH), 114.0 (C), 113.6 (C), 52.6 (CH_3), 52.6 (CH_3), 52.5 (CH_3), 52.3 (CH_3); LRMS $[M + Na]^+$ 385.07; HRMS calcd for $C_{18}H_{15}O_6ClNa$: *M*Na^+, 385.0455. Found: *M*Na^+, 385.0445.

Methyl 3-(1-(2-chlorophenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 15. Phosphonate **10** (150 mg, 0.44 mmol) and *o*-chlorobenzaldehyde (97 μL, 0.87 mmol) were reacted for 16 h at −78 °C according to Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 90 : 10 v/v) to afford diester **15** as a mixture of diastereomers as a colourless oil (89 mg, 57%, 91 : 9 *Z*/*E*); IR ν_{max} (ATR): 3090 (br), 2954, 1729, 1677, 1465, 1438, 1249 cm^{-1}; *Z*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.33; ^1H NMR (400 MHz, $CDCl_3$) δ 11.06 (1H, s, OH), 8.07 (1H, app. d, *J* 7.4 Hz, Ar-H), 7.75 (1H, s, C═C*H*Ph), 7.52 (1H, d, *J* 8.1 Hz, Ar-H), 7.38 (1H, app. d, *J* 7.4 Hz, H-6), 7.20 (2H, app. quintet, *J* 7.3 Hz, Ar-H), 6.98 (1H, app. d, *J* 7.9 Hz, H-4), 6.73 (1H, app. t, *J* 7.9 Hz, H-5), 3.94 (3H, s, CO_2Me), 3.77 (3H, s, CO_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 (C═O), 163.3 (C═O), 151.8 (C), 144.8 (C), 141.3 (C), 134.5 (CH), 130.9 (C), 130.3 (CH), 130.3

(CH), 129.5 (CH), 126.9 (C), 123.7 (CH), 121.9 (CH), 120.3 (CH), 118.3 (CH), 113.5 (C) 52.6 (CH_3), 52.4 (CH_3); *E*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.30; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.04 (1H, s, OH), 7.67 (1H, dd, *J* 1.5, 8.1 Hz, H-6), 7.38–7.36 (1H, m, Ar-H), 7.33–7.31 (2H, m, Ar-H), 7.24–7.18 (2H, m, Ar-H + H-4), 6.87 (1H, app. t, *J* 8.0 Hz, H-5), 6.54 (1H, s, C═C*H*Ph), 3.97 (3H, s, CO_2Me), 3.67 (3H, s, CO_2Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4 (C═O), 162.7 (C═O), 153.0 (C), 145.0 (C), 143.9 (C), 133.3 (C), 132.6 (CH), 130.7 (CH), 129.1 (CH), 129.0 (CH), 126.2 (CH), 125.5 (C), 125.2 (C), 119.1 (CH), 118.8 (CH), 114.1 (C) 52.5 (CH_3), 52.2 (CH_3); LRMS $[M + Na]^+$ 385.07; HRMS calcd for $C_{18}H_{15}O_6ClNa$: MNa$^+$, 385.0455. Found: MNa$^+$, 385.0447.

Methyl 3-(1-(4-chlorophenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 16. Phosphonate **10** (150 mg, 0.44 mmol) and *p*-chlorobenzaldehyde (120 mg, 0.87 mmol) were reacted for 2 h at −78 °C according to Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 90 : 10 v/v) to afford diester **16** as a mixture of diastereomers as a yellow oil (110 mg, 70%, 67 : 33 *Z*/*E*). IR ν_{max} (ATR): 3136 (br), 2957, 1727, 1677, 1464, 1438, 1249 cm^{-1}; *Z*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.34; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.06 (1H, s, OH), 7.68 (1H, app. d, *J* 8.6 Hz, Ar-H), 7.55 (1H, dd, *J* 1.4, 8.1 Hz, H-6), 7.31 (1H, s, C═C*H*Ph), 7.29–7.26 (3H, m, Ar-H), 6.96 (1H, dd, *J* 1.0, 8.0 Hz, H-4), 6.73 (1H, app. t, *J* 8.0 Hz, H-5), 3.97 (3H, s, CO_2Me), 3.75 (3H, s, CO_2Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5 (C═O), 163.6 (C═O), 151.9 (C), 144.7 (C), 140.5 (C), 135.6 (C), 131.6 (2 × CH), 129.0 (2 × CH), 128.2 (CH), 125.6 (CH), 123.7 (CH), 120.1 (CH), 118.4 (CH), 113.7 (C), 52.5 (CH_3), 52.5 (CH_3); *E*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.31; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.01 (1H, s, OH), 7.67 (1H, app. d, *J* 8.9 Hz, Ar-H), 7.66 (1H, dd, *J* 1.5, 8.1 Hz, H-6), 7.29–7.26 (3H, m, Ar-H), 7.25 (1H, dd, *J* 1.5, 8.0 Hz, H-4), 6.86 (1H, app. t, *J* 8.0 Hz, H-5), 6.44 (1H, s, C═C*H*Ph), 3.97 (3H, s, CO_2Me), 3.72 (3H, s, CO_2Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4 (C═O), 163.1 (C═O), 152.9 (C), 143.8 (C), 143.8 (C) 133.8 (C), 131.7 (2 × CH), 130.8 (2 × CH), 130.3 (CH), 125.5 (CH), 125.0 (CH), 120.5 (CH), 118.7 (CH), 114.1 (C), 52.6 (CH_3), 52.3 (CH_3); LRMS $[M + Na]^+$ 385.33; HRMS calcd for $C_{18}H_{15}O_6ClNa$: MNa$^+$, 385.0455. Found: MNa$^+$, 385.0449.

Methyl 2-hydroxy-3-(3-methoxy-3-oxo-1-*m*-tolylprop-1-en-2-yloxy)benzoate 17. Phosphonate **10** (70 mg, 0.20 mmol) and *m*-tolualdehyde (47 µL, 0.40 mmol) were reacted for 2 h at −78 °C according to Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 90 : 10 v/v) to afford diester **17** as a mixture of diastereomers as a yellow oil (56 mg, 82%, 56 : 44 *Z*/*E*). R_f [hexane : EtOAc 5 : 1 v/v] = 0.31; IR ν_{max} (ATR): 3099 (br), 2953, 1727, 1677, 1438, 1249 cm^{-1}; *Z*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.10 (1H, s, OH), 7.57 (1H, app d, *J* 7.8 Hz, Ar-H), 7.53 (1H, s, C═C*H*Ph), 7.37 (1H, s, Ar-H), 7.26–7.08 (3H, m, Ar-H + H-6), 6.96 (1H, dm, *J* 8.0 Hz, H-4), 6.72 (1H, t, *J* 8.0 Hz, H-5), 3.96 (3H, s, CO_2Me), 3.76 (3H, s, CO_2Me), 2.31 (3H, s, Ar-*Me*); *E*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.04 (1H, s, OH), 7.63 (1H, dm, *J* 8.1 Hz, Ar-H), 7.52 (1H, dm, *J* 8.0 Hz, H-6), 7.26–7.08 (4H, m, Ar-H + H-4), 6.84 (1H, t, *J* 8.0 Hz, H-5), 6.56 (1H, s, C═C*H*Ph), 3.96 (3H, s, CO_2Me), 3.70 (3H, s, CO_2Me), 2.33 (3H, s, Ar-*Me*); (*E* + *Z* mix) ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C═O), 170.3 (C═O), 163.8 (C═O), 163.3 (C═O), 152.7 (C), 151.8 (C), 144.9 (C), 144.2 (C), 142.9 (C), 139.6 (C), 138.2 (C), 137.6 (C), 132.9 (C), 132.1 (C), 131.2 (C), 130.6 (C), 129.5 (CH), 128.8 (CH), 128.6 (CH), 127.9 (CH), 127.6 (CH), 127.4 (CH), 125.9 (CH), 125.0 (CH), 124.3 (CH), 123.3 (CH), 122.7 (CH), 119.7 (CH), 118.6 (CH), 118.3 (CH), 113.8 (C), 113.4 (C), 52.5 (CH_3), 52.4 (CH_3), 52.4 (CH_3), 52.2 (CH_3), 21.3 (CH_3), 21.3 (CH_3); LRMS $[M + Na]^+$ 365.13; HRMS calcd for $C_{19}H_{18}O_6Na$: MNa$^+$, 365.0996. Found: MNa$^+$, 365.0990.

Methyl 2-hydroxy-3-(3-methoxy-3-oxo-1-*o*-tolylprop-1-en-2-yloxy)benzoate 18. Phosphonate **10** (150 mg, 0.44 mmol) and *o*-tolualdehyde (100 µL, 0.87 mmol) were reacted for 3 h at −78 °C according to Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 90 : 10 v/v) to afford diester **18** as a mixture of diastereomers as a colourless oil (88 mg, 60%, 91 : 9 *Z* : *E*) IR ν_{max} (ATR): 3125 (br), 2954, 1728, 1676, 1465, 1438, 1248 cm^{-1}; *Z*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.26; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.06 (1H, s, OH), 7.91 (1H, app. d, *J* 7.8 Hz, Ar-H), 7.60 (1H, s, C═C*H*Ph), 7.50 (1H, dd, *J* 1.5, 8.1 Hz, Ar-H), 7.25 (1H, dd, *J* 1.5, 7.3 Hz, H-6), 7.22–7.12 (2H, m, Ar-H), 6.95 (1H, dd, *J* 1.5, 8.0 Hz, H-4), 6.72 (1H, app. t, *J* 8.0 Hz, H-5), 3.96 (3H, s, CO_2Me), 3.77 (3H, s, CO_2Me), 2.45 (3H, s, Ar-*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C═O), 162.9 (C═O), 151.8 (C), 145.1 (C), 140.0 (C), 135.9 (CH), 130.2 (C), 129.5 (CH), 128.8 (CH), 126.2 (CH), 125.2 (C), 124.3 (CH), 124.1 (CH), 123.3 (CH), 119.9 (CH), 113.4 (C) 52.5 (CH_3), 52.4 (CH_3), 20.0 (CH_3); *E*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.18; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.04 (1H, s, OH), 7.65 (1H, dd, *J* 1.6, 8.1 Hz, H-6), 7.22–7.12 (5H, m, Ar-H + H-4), 6.87 (1H, app. t, *J* 8.0 Hz, H-5), 6.61 (1H, s, C═C*H*Ph), 3.98 (3H, s, CO_2Me), 3.64 (3H, s, CO_2Me), 2.25 (3H, s, Ar-*Me*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3 (C═O), 163.8 (C═O), 152.8 (C), 144.1 (C), 143.2 (C), 137.6 (C), 132.8 (CH), 130.8 (CH), 129.5 (CH), 129.4 (CH), 127.9 (CH), 125.0 (C), 122.2 (C), 118.7 (CH), 118.3 (CH), 113.8 (C) 52.5 (CH_3), 52.1 (CH_3), 20.2 (CH_3); LRMS $[M + Na]^+$ 365.07; HRMS calcd for $C_{19}H_{18}O_6Na$: MNa$^+$, 365.0996. Found: MNa$^+$, 365.0989.

Methyl 2-hydroxy-3-(3-methoxy-3-oxo-1-*p*-tolylprop-1-en-2-yloxy)benzoate 19. Phosphonate **10** (140 mg, 0.41 mmol) and *p*-tolualdehyde (100 µL, 0.83 mmol) were reacted for 3 h at −78 °C under Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 5 : 1 v/v) to afford diester **19** as a mixture of diastereomers as a white solid (71 mg, 50%, 67 : 33 *Z*/*E*). R_f [hexane : EtOAc 5 : 1 v/v] = 0.38; IR ν_{max} (ATR): 3137 (br), 2954, 2923, 1726, 1678, 1464, 1439, 1249 cm^{-1}; *Z*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.05 (1H, s, OH), 7.64 (2H, d, *J* 8.1 Hz, Ar-H), 7.52 (1H, dd, *J* 1.5, 8.0 Hz, H-6), 7.38 (1H, s, C═C*H*Ph), 7.14 (2H, d, *J* 8.1 Hz, Ar-H), 6.96 (1H, dd, *J* 1.5, 8.0 Hz, H-4), 6.71 (1H, app. t, *J* 8.0 Hz, H-5), 3.96 (3H, s, CO_2Me), 3.75 (3H, s, CO_2Me), 2.32 (3H, s, Ar-*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (C═O), 164.0 (C═O), 152.0 (C), 145.1 (C), 140.3 (C), 139.2 (C), 130.6 (2 × CH), 129.6 (2 × CH), 127.8 (CH), 123.5 (CH), 119.8 (CH), 118.5 (CH), 113.7 (C), 52.6 (CH_3), 52.6 (CH_3), 21.6 (CH_3) 1 signal obscured; *E*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.00 (1H, s,

OH), 7.62 (1H, dd, *J* 1.5, 8.0 Hz, H-6), 7.26 (2H, d, *J* 8.2 Hz, Ar-H), 7.24–7.21 (1H, m, H-4), 7.14–7.11 (2H, m, Ar-H), 6.83 (1H, app. t, *J* 8.0 Hz, H-5), 6.59 (1H, s, C=C*H*Ph), 3.96 (3H, s, CO_2Me), 3.70 (3H, s, CO_2Me), 2.34 (3H, s, Ar-*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C=O), 163.5 (C=O), 152.8 (C), 144.7 (C), 142.4 (C), 138.3 (C), 130.1 (C), 129.1 (2 × CH), 128.9 (2 × CH), 124.9 (CH), 124.1 (CH), 123.8 (CH), 118.8 (CH), 114.0 (C), 52.7 (CH_3), 52.3 (CH_3), 21.4 (CH_3); LRMS $[M + Na]^+$ 364.87; HRMS calcd for $C_{19}H_{18}O_6Na$: $M Na^+$, 365.1001. Found: $M Na^+$, 365.0994.

Methyl 3-(1-(3-acetoxyphenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 20. Phosphonate **10** (150 mg, 0.43 mmol) and *m*-formylphenyl acetate (140 mg, 0.86 mmol) were reacted for 6 h at −78 °C under Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 5 : 1 v/v) to afford diester **20** as a mixture of diastereomers as a yellow oil (94 mg, 56%, 41 : 59 *Z*/*E*). R_f [hexane : EtOAc 5 : 1 v/v] = 0.21; IR ν_{max} (ATR): 3046 (br), 2954, 1765, 1729, 1677, 1439, 1199 cm^{-1}; *Z*: ^1H NMR (400 MHz, $CDCl_3$) δ 11.06 (1H, s, OH), 7.61 (1H, app. d, *J* 7.8 Hz, Ar-H), 7.54 (1H, dd, *J* 1.4, 8.0 Hz, H-6), 7.48 (1H, app. t, *J* 2.2 Hz, Ar-H), 7.34 (1H, t, *J* 8.0 Hz, Ar-H), 7.32 (1H, s, C=C*H*Ph), 7.08 (1H, ddd, *J* 1.0, 2.2, 7.8 Hz, Ar-H), 6.97 (1H, dd, *J* 1.4, 8.0 Hz, H-4), 6.73 (1H, t, *J* 8.0 Hz, H-5), 3.97 (3H, s, CO_2Me), 3.75 (3H, s, CO_2Me), 2.27 (3H, s, COMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (C=O), 169.3 (C=O), 163.2 (C), 152.1 (C), 150.8 (C), 144.9 (C), 141.0 (C), 133.9 (C), 129.7 (CH), 127.9 (CH), 125.8 (CH), 123.9 (CH), 123.4 (CH), 123.0 (CH), 120.5 (CH), 118.5 (CH), 113.7 (C), 52.6 (CH_3), 52.4 (CH_3), 21.2 (CH_3); *E*: ^1H NMR (400 MHz, $CDCl_3$) δ 11.01 (1H, s, OH), 7.66 (1H, dd, *J* 1.6, 8.0 Hz, H-6), 7.31 (1H, t, *J* 7.8 Hz, Ar-H), 7.25 (1H, dd, *J* 1.6, 8.0 Hz, H-4), 7.16 (1H, dt, *J* 0.8, 1.9, 7.8 Hz, Ar-H), 7.08 (1H, app. t, *J* 1.9 Hz, Ar-H), 6.99 (1H, ddd, *J* 0.8, 1.9, 7.8 Hz, Ar-H), 6.85 (1H, t, *J* 8.0 Hz, H-5), 6.45 (1H, s, C=C*H*Ph), 3.96 (3H, s, CO_2Me), 3.71 (3H, s, CO_2Me), 2.28 (3H, s, COMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4 (C=O), 169.4 (C=O), 163.6 (C), 153.1 (C), 150.4 (C), 144.3 (C), 143.8 (C), 134.8 (C), 129.0 (CH), 126.6 (CH), 125.6 (CH), 125.3 (CH), 122.3 (CH), 121.1 (CH), 120.3 (CH), 118.8 (CH), 114.1 (C), 52.6 (CH_3), 52.6 (CH_3), 21.2 (CH_3); LRMS $[M + Na]^+$ 408.87; HRMS calcd for $C_{20}H_{18}O_8Na$: $M Na^+$, 409.0899. Found: $M Na^+$, 409.0896.

Methyl 3-(1-(2-acetoxyphenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 21. Phosphonate **10** (150 mg, 0.43 mmol) and *o*-formylphenyl acetate (140 mg, 0.86 mmol) were reacted for 6 h at −78 °C under Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 5 : 1 v/v) to afford diester **21** as a mixture of diastereomers as a yellow oil (99 mg, 60%, 67 : 33 *Z*/*E*). R_f [hexane : EtOAc 5 : 1 v/v] = 0.31; IR ν_{max} (ATR): 3094 (br), 2952, 1768, 1731, 1678, 1464, 1251 cm^{-1}; *Z*: ^1H NMR (400 MHz, $CDCl_3$) δ 11.04 (1H, s, OH), 8.11 (1H, dd, *J* 1.6, 8.0 Hz, Ar-H), 7.52 (1H, dd, *J* 1.6, 8.0 Hz, H-6), 7.43 (1H, s, C=C*H*Ph), 7.35–7.31 (1H, m, Ar-H), 7.18 (1H, dt, *J* 0.6, 7.2, 7.8 Hz, Ar-H), 7.09 (1H, dd, *J* 0.6, 7.8 Hz, Ar-H), 6.95 (1H, dd, *J* 1.6, 8.0 Hz, H-4), 6.72 (1H, t, *J* 8.0 Hz, H-5), 3.96 (3H, s, CO_2Me), 3.75 (3H, s, CO_2Me), 2.41 (3H, s, COMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (C=O), 169.3 (C=O), 163.7 (C), 152.1 (C), 149.2 (C), 145.0 (C), 141.4 (C), 130.7 (C), 130.7 (CH), 126.5 (CH), 125.2 (CH), 123.8 (CH), 122.5 (CH), 120.5 (CH), 119.5 (CH), 118.6 (CH), 113.7 (C), 52.7 (CH_3), 52.6 (CH_3), 21.1 (CH_3); *E*: ^1H NMR (400 MHz, $CDCl_3$) δ 11.02 (1H, s, OH), 7.68 (1H, dd, *J* 1.3, 8.0 Hz, H-6), 7.31–7.27 (2H, m, Ar-H), 7.26 (1H, dd, *J* 1.4, 8.0 Hz, Ar-H), 7.19 (1H, dt, *J* 0.8, 7.6, 8.0 Hz, Ar-H), 7.06 (1H, dd, *J* 1.4, 8.0 Hz, H-4) 6.87 (1H, t, *J* 8.0 Hz, H-5), 6.20 (1H, s, C=C*H*Ph), 3.97 (3H, s, CO_2Me), 3.69 (3H, s, CO_2Me), 2.23 (3H, s, COMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C=O), 169.1 (C=O), 163.0 (C), 153.3 (C), 148.2 (C), 145.5 (C), 143.4 (C), 130.4 (CH), 129.0 (CH), 127.2 (CH), 127.2 (C), 126.0 (CH), 125.8 (CH), 122.2 (CH), 119.0 (CH), 114.5 (CH), 114.3 (C), 52.7 (CH_3), 52.4 (CH_3), 20.9 (CH_3); LRMS $[M + Na]^+$ 408.87; HRMS calcd for $C_{20}H_{18}O_8Na$: $M Na^+$, 409.0899. Found: $M Na^+$, 409.0895.

Methyl 3-(1-(4-acetoxyphenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 22. Phosphonate **10** (150 mg, 0.43 mmol) and *p*-formylphenyl acetate (140 mg, 0.86 mmol) were reacted for 6 h at −78 °C under Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 5 : 1 v/v) to afford diester **22** as a mixture of diastereomers as a yellow oil (100 mg, 61%, 56 : 44 *Z*/*E*). IR ν_{max} (ATR): 3144 (br), 2957, 1765, 1727, 1677, 1463, 1439, 1195 cm^{-1}; *Z*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.45, ^1H NMR (400 MHz, $CDCl_3$) δ 11.06 (1H, s, OH), 7.77 (2H, d, *J* 8.6 Hz, Ar-H), 7.53 (1H, dd, *J* 1.3, 8.1 Hz, H-6), 7.36 (1H, s, C=C*H*Ph), 7.07 (2H, d, *J* 8.6 Hz, Ar-H), 6.95 (1H, dd, *J* 1.3, 8.1 Hz, H-4), 6.72 (1H, t, *J* 8.1 Hz, H-5), 3.96 (3H, s, CO_2Me), 3.75 (3H, s, CO_2Me), 2.28 (3H, s, COMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (C=O), 169.4 (C=O), 163.9 (C), 152.1 (C), 151.6 (C), 145.0 (C), 140.1 (C), 131.8 (2 × CH), 130.2 (CH), 126.3 (CH), 123.8 (CH), 122.1 (2 × CH), 120.1 (CH), 118.6 (CH), 113.8 (C), 52.7 (CH_3), 52.6 (CH_3), 21.3 (CH_3); *E*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.36; ^1H NMR (400 MHz, $CDCl_3$) δ 11.01 (1H, s, OH), 7.64 (1H, dd, *J* 1.5, 8.0 Hz, H-6), 7.36 (2H, d, *J* 7.8 Hz, Ar-H), 7.23 (1H, dd, *J* 1.5, 8.0 Hz, H-4), 7.04 (2H, d, *J* 7.8 Hz, Ar-H), 6.85 (1H, t, *J* 8.0 Hz, H-5), 6.52 (1H, s, C=C*H*Ph), 3.96 (3H, s, CO_2Me), 3.70 (3H, s, CO_2Me), 2.29 (3H, s, COMe); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C=O), 169.3 (C=O), 163.4 (C), 153.0 (C), 150.5 (C), 144.3 (C), 143.5 (C), 130.9 (CH), 130.4 (2 × CH), 125.4 (CH), 124.7 (CH), 121.8 (CH), 121.4 (2 × CH), 118.9 (CH), 114.1 (C), 52.6 (CH_3), 52.4 (CH_3), 21.2 (CH_3); LRMS $[M + Na]^+$ 408.87; HRMS calcd for $C_{20}H_{18}O_8Na$: $M Na^+$, 409.0899. Found: $M Na^+$, 409.0896.

Methyl 3-(1-(3-trifluoromethylphenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 23. Phosphonate **10** (150 mg, 0.43 mmol) and *m*-trifluoromethylbenzaldehyde (140 mg, 0.86 mmol) were reacted for 6 h at −78 °C according to Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 90 : 10 v/v) to afford diester **23** as a mixture of diastereomers as a yellow oil (114 mg, 67%, 59 : 41 *Z*/*E*). R_f [hexane : EtOAc 5 : 1 v/v] = 0.33; IR ν_{max} (ATR): 3117 (br), 2957, 1730, 1678, 1439, 1327, 1121 cm^{-1}; *Z*: ^1H NMR (400 MHz, $CDCl_3$) δ 11.10 (1H, s, OH), 7.97 (1H, app. d, *J* 7.9 Hz, Ar-H), 7.94 (1H, s, C=C*H*Ph), 7.56–7.40 (2H, m, H-6 + H-4), 7.48 (1H, app. t, *J* 2.2 Hz, Ar-H), 7.35 (1H, app. s, Ar-H), 6.97 (1H, app. d, *J* 7.7 Hz, Ar-H), 6.73 (1H, t, *J* 8.1 Hz,

H-5), 3.96 (3H, s, CO_2Me), 3.77 (3H, s, CO_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C═O), 163.3 (C═O), 151.9 (C), 144.5 (C), 141.4 (C), 134.1 (CH), 133.1 (C), 131.0 (C, q, $^2J_{C-F}$ 32.7 Hz), 127.1 (CH, q, $^3J_{C-F}$ 3.8 Hz), 127.1 (CH, q, $^3J_{C-F}$ 3.7 Hz), 125.7 (CH), 123.9 (CH), 123.7 (C, q, $^1J_{C-F}$ 272 Hz), 120.2 (CH), 118.4 (CH), 113.6 (C), 52.6 (CH_3), 52.5 (CH_3) 1 signal obscured; *E*: ^1H NMR (400 MHz, $CDCl_3$) δ 11.07 (1H, s, OH), 7.67 (1H, dd, *J* 1.0, 8.1 Hz, H-6), 7.56–7.40 (4H, m, Ar-H), 7.27 (1H, app. d, *J* 7.9 Hz, H-4), 6.88 (1H, t, *J* 8.0 Hz, H-5), 6.42 (1H, s, C═C*H*Ph), 3.96 (3H, s, CO_2Me), 3.72 (3H, s, CO_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (C═O), 162.9 (C═O), 153.0 (C), 144.8 (C), 143.2 (C), 133.0 (C), 132.1 (CH), 130.3 (C, q, $^2J_{C-F}$ 35.3 Hz), 125.9 (CH), 125.7 (CH), 125.7 (CH, q, $^3J_{C-F}$ 4.9 Hz), 124.8 (CH), 124.3 (CH, q, $^3J_{C-F}$ 3.8 Hz), 123.9 (C, q, $^1J_{C-F}$ 272 Hz), 118.8 (CH), 118.7 (CH), 114.1 (C), 52.6 (CH_3), 52.3 (CH_3); LRMS $[M + Na]^+$ 418.67; HRMS calcd for $C_{19}H_{15}O_6F_3Na$: MNa$^+$, 419.0713. Found: MNa$^+$, 419.0719.

Methyl 3-(1-(2-trifluoromethylphenyl)-3-methoxy-3-oxoprop-1-en-2-yloxy)-2-hydroxybenzoate 24. Phosphonate **10** (150 mg, 0.44 mmol) and *o*-trifluoromethylbenzaldehyde (100 μL, 0.87 mmol) were reacted for 16 h at −78 °C according to Procedure A. The product was purified by column chromatography (eluent: hexane–EtOAc 90 : 10 v/v) to afford diester **24** as a mixture of diastereomers as a colourless oil (115 mg, 67%, 91 : 9 *Z* : *E*) IR ν_{max} (ATR): 3099 (br), 2956, 1732, 1677, 1464, 1439, 1249 cm^{-1}; *Z*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.27; ^1H NMR (400 MHz, $CDCl_3$) δ 11.06 (1H, s, OH), 8.07 (1H, app. d, *J* 7.7 Hz, Ar-H), 7.65 (1H, app. d, *J* 7.6 Hz, Ar-H) 7.65 (1H, s, C═C*H*Ph), 7.52 (1H, app. d, *J* 8.5 Hz, H-6), 7.46 (1H, app. d, *J* 7.7 Hz, Ar-H), 7.37 (1H, app. d, *J* 7.6 Hz, Ar-H), 6.95 (1H, app. d, *J* 7.9 Hz, H-4), 6.72 (1H, app. t, *J* 8.0 Hz, H-5), 3.94 (3H, s, CO_2Me), 3.78 (3H, s, CO_2Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5 (C═O), 163.2 (C═O), 151.8 (C), 144.9 (C), 141.9 (C), 131.8 (CH), 131.1 (C), 130.3 (C, q, $^3J_{C-F}$ 2.4 Hz), 128.8 (CH), 128.5 (C, q, $^2J_{C-F}$ 29.7 Hz), 125.8 (CH, q, $^3J_{C-F}$ 5.6 Hz), 123.9 (C, q, $^1J_{C-F}$ 274 Hz), 123.8 (CH), 121.4 (CH), 120.5 (CH), 118.4 (CH), 113.5 (C), 52.7 (CH_3), 52.5 (CH_3); *E*: R_f [hexane : EtOAc 5 : 1 v/v] = 0.25; ^1H NMR (500 MHz, $CDCl_3$) δ 11.02 (1H, s, OH), 7.68–7.65 (2H, m, Ar-H + H-6), 7.49 (1H, app. t, *J* 8.3 Hz, Ar-H), 7.39 (1H, app. t, *J* 7.5 Hz, Ar-H), 7.32 (1H, app. d, *J* 7.6 Hz, H-4), 7.27 (1H, dd, *J* 1.4, 7.6 Hz, Ar-H), 6.88 (1H, app. t, *J* 8.0 Hz, H-5), 6.64 (1H, s, C═C*H*Ph), 3.98 (3H, s, CO_2Me), 3.61 (3H, s, CO_2Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4 (C═O), 162.4 (C═O), 153.0 (C), 144.9 (C), 143.6 (C), 132.9 (C, q, $^3J_{C-F}$ 2.2 Hz), 131.2 (CH), 130.8 (CH), 127.9 (C, q, $^2J_{C-F}$ 29.9 Hz), 127.6 (CH), 125.7 (CH), 125.6 (CH, q, $^3J_{C-F}$ 5.4 Hz), 125.4 (C), 121.8 (C, q, $^1J_{C-F}$ 290 Hz), 118.8 (CH), 118.3 (CH), 114.1 (C) 52.5 (CH_3), 52.2 (CH_3); LRMS $[M + Na]^+$ 418.9; HRMS calcd for $C_{19}H_{15}O_6F_3Na$: MNa$^+$, 419.0713. Found: MNa$^+$, 419.0718.

Procedure B: saponification reaction of dimethyl esters

Aqueous potassium hydroxide (2 M soln., 20 eq.) was added to a solution of diester in THF (1 mL). Water was then added to make the solution up to 2 mL (or 2.6 mL when the base volume exceeded 1 mL) and the reaction stirred at rt for 48 h. Work-up was carried out using one of two methods; Method A: THF removed *in vacuo* prior to acidification to pH 5–6 with 1 M HCl and extraction into DCM (3 × 10 mL). The organic layers were dried (Na_2SO_4), and solvent removed *in vacuo* to afford the desired acids. Method B: THF removed *in vacuo* prior to acidification to pH 5–6 with 1 M HCl. The solution was filtered and purified immediately using reverse phase HPLC (gradient: 0–100% MeCN with 0.1% formic acid, from 100% H_2O with 0.1% formic acid over 40 minutes). The fractions containing the desired diacid were lyophilised.

3-(2-(3-Bromophenyl)-1-carboxyvinyloxy)-2-hydroxybenzoic acid 25. Potassium hydroxide (1.2 mL) was added to a solution of diester **11** (48 mg, 0.12 mmol) in THF (1.5 mL). Water (0.60 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked up according to Method A to afford the desired diacid **25** as a mixture of diastereomers as a colourless oil (24 mg, 54%, 63 : 37 *Z* : *E*). IR ν_{max} (ATR): 3075 (br), 2570, 1686, 1467, 1237 cm^{-1}; *Z*: ^1H NMR (400 MHz, MeOD) δ 7.95 (1H, s, C═C*H*Ph), 7.68 (1H, app. d, *J* 7.8 Hz, Ar-H), 7.55 (1H, dd, *J* 1.6, 8.0 Hz, H-6), 7.45 (1H, dm, *J* 8.0 Hz, Ar-H), 7.32 (1H, s, Ar-H), 7.24 (1H, t, *J* 8.0 Hz, Ar-H), 6.98 (1H, dd, *J* 1.4, 8.0 Hz, H-4), 6.75 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (100 MHz, MeOD) δ 173.6 (C═O), 165.7 (C═O), 153.2 (C), 146.0 (C), 143.1 (C), 136.2 (C), 133.8 (C), 133.4 (CH), 132.7 (CH), 130.0 (CH), 125.7 (CH), 125.2 (CH), 123.6 (CH), 120.9 (CH), 119.3 (CH), 115.2 (C); *E*: ^1H NMR (400 MHz, MeOD) δ 7.69 (1H, dd, *J* 1.6, 8.0 Hz, Ar-H), 7.51 (1H, br t, *J* 5.0 Hz, Ar-H), 7.38 (2H, dm, *J* 7.8 Hz, Ar-H + H-6), 7.27 (1H, dd, *J* 1.6, 8.0 Hz, H-4), 7.18 (1H, t, *J* 7.8 Hz, Ar-H), 6.89 (1H, t, *J* 8.0 Hz, H-5), 6.33 (1H, s, C═C*H*Ph); ^{13}C NMR (100 MHz, MeOD) δ 173.4 (C═O), 165.7 (C═O), 154.5 (C), 146.9 (C), 144.7 (C), 137.4 (C), 131.4 (C), 131.4 (CH), 130.7 (CH), 128.9 (CH), 127.3 (CH), 126.4 (CH), 122.8 (CH), 119.7 (CH), 118.9 (CH), 115.8 (C); LRMS $[M + Na]^+$ 400.53; HRMS calcd for $C_{16}H_{11}O_6BrNa$: MNa$^+$, 400.9637. Found: MNa$^+$, 400.9636.

***Z*-3-(2-(2-Bromophenyl)-1-carboxyvinyloxy)-2-hydroxybenzoic acid 26.** Potassium hydroxide (0.17 mL) was added to a solution of pure *Z* diester **12** (7 mg, 0.02 mmol) in THF. Water (0.83 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method B, affording the desired pure *Z* diacid **26** as a white solid (7 mg, quant). IR ν_{max} (ATR): 3081 (br), 2578, 1682, 1466, 1237, 753 cm^{-1}; retention time: 33.53 min; mp 211–213 °C; ^1H NMR (300 MHz, MeOD) δ 8.00 (1H, dd, *J* 1.7, 7.8 Hz, Ar-H), 7.67 (1H, s, C═C*H*Ph), 7.63 (1H, dd, *J* 1.1, 7.9 Hz, Ar-H), 7.53 (1H, dd, *J* 1.5, 8.0 Hz, H-6), 7.29 (1H, dt, *J* 1.1, 7.6, 7.9 Hz, Ar-H), 7.19 (1H, dt, *J* 1.7, 7.6, 7.8 Hz, Ar-H), 6.98 (1H, dd, *J* 1.5, 8.0 Hz, H-4), 6.74 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (75 MHz, MeOD) δ 173.7 (C═O), 165.9 (C═O), 153.2 (C), 146.3 (C), 143.8 (C), 134.0 (CH), 133.8 (C), 132.1 (CH), 131.7 (CH), 128.7 (CH), 125.7 (C), 125.2 (CH), 124.7 (CH), 121.0 (CH), 119.1 (CH), 115.9 (C); LRMS $[M + Na]^+$ 402.60; HRMS calcd for $C_{16}H_{11}O_6BrNa$: MNa$^+$, 400.9637. Found: MNa$^+$, 400.9628.

***Z*-3-(2-(4-Bromophenyl)-1-carboxyvinyloxy)-2-hydroxybenzoic acid 27.** Potassium hydroxide (0.29 mL) was added to a solution of pure *Z* diester **13** (12 mg, 0.03 mmol) in THF. Water (0.71 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method B, affording

the desired pure *Z* diacid **27** as a white solid (9 mg, 79%). IR ν_{max} (ATR): 3097 (br), 2571, 1686, 1466, 1240, 753 cm^{-1}; retention time: 33.50 min; ^1H NMR (300 MHz, MeOD) δ 7.67 (2H, d, *J* 8.6 Hz, Ar-H), 7.55 (1H, dd, *J* 1.5, 8.0 Hz, H-6), 7.51 (2H, d, *J* 8.6 Hz, Ar-H), 7.34 (1H, s, C═C*H*Ph), 6.98 (1H, dd, *J* 1.5, 8.0 Hz, H-4), 6.75 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (75 MHz, MeOD) δ 173.8 (C═O), 166.0 (C═O), 153.2 (C), 146.1 (C), 146.1 (C), 142.8 (C), 133.3 (C), 132.9 (2 × CH), 132.9 (2 × CH), 126.0 (CH), 125.2 (CH), 124.5 (C), 120.6 (CH), 119.1 (CH); LRMS $[M - H]^-$ 376.73; HRMS calcd for $C_{16}H_{11}O_6BrNa$: *M*Na^+, 400.9637. Found: *M*Na^+, 400.9628.

3-(2-(3-Chlorophenyl)-1-carboxyvinyloxy)-2-hydroxybenzoic acid 28. Potassium hydroxide (1.4 mL) was added to a solution of diester **14** (50 mg, 0.14 mmol) in THF (1.5 mL). Water (0.70 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked up according to Method A, affording the desired diacid **28** as a mixture of diastereomers as a colourless oil (31 mg, 68%, 59 : 41 *Z* : *E*). IR ν_{max} (ATR): 3064 (br), 2582, 1682, 1466, 1232 cm^{-1}; *Z*: ^1H NMR (400 MHz, MeOD) δ 7.80 (1H, s, C═C*H*Ph), 7.54 (1H, app. d, *J* 8.0 Hz, Ar-H), 7.37–7.22 (4H, m, Ar-H + H-6), 6.98 (1H, app. d, *J* 8.0 Hz, H-4), 6.76 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (100 MHz, MeOD) δ 173.6 (C═O), 165.7 (C═O), 153.3 (C), 146.0 (C), 143.1 (C), 136.0 (C), 135.6 (C), 131.2 (CH), 130.8 (CH), 129.6 (CH), 128.5 (CH), 125.7 (CH), 125.2 (CH), 121.0 (CH), 119.3 (CH), 115.2 (C); *E*: ^1H NMR (400 MHz, MeOD) δ 7.69 (1H, app. d, *J* 8.0 Hz, Ar-H), 7.65 (1H, br t, *J* 3.7 Hz, Ar-H), 7.37–7.22 (4H, m, Ar-H + H-6 + H-4), 6.90 (1H, t, *J* 8.0 Hz, H-5), 6.34 (1H, s, C═C*H*Ph); ^{13}C NMR (100 MHz, MeOD) δ 173.4 (C═O), 165.8 (C═O), 154.6 (C), 147.0 (C), 144.8 (C), 137.2 (C), 134.8 (C), 130.5 (CH), 130.4 (CH), 129.8 (CH), 128.5 (CH), 127.3 (CH), 126.4 (CH), 119.7 (CH), 118.9 (CH), 115.8 (C); LRMS $[M + Na]^+$ 357.07; HRMS calcd for $C_{16}H_{11}O_6ClNa$: *M*Na^+, 357.0136. Found: *M*Na^+, 357.0137.

***Z*-3-(2-(2-Chlorophenyl)-1-carboxyvinyloxy)-2-hydroxybenzoic acid 29.** Potassium hydroxide (0.5 mL) was added to a solution of pure *Z* diester **15** (13.8 mg, 0.04 mmol) in THF (0.5 mL). Water (0.25 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method B, affording the desired pure *Z* diacid **29** as a white solid (13.1 mg, quant). IR ν_{max} (ATR): 3214 (br), 2567, 1668, 1467, 1240, 1054 cm^{-1}; retention time: 33.53 min; ^1H NMR (400 MHz, MeOD) δ 8.04 (1H, dd, *J* 1.8, 7.7 Hz, Ar-H), 7.72 (1H, s, C═C*H*Ph), 7.53 (1H, dd, *J* 1.4, 8.0 Hz, Ar-H), 7.44 (1H, dd, *J* 1.3, 8.0 Hz, H-6), 7.29 (1H, dt, *J* 1.8, 7.4 Hz, Ar-H), 7.24 (1H, dt, *J* 1.1, 7.5 Hz, Ar-H), 6.98 (1H, dd, *J* 1.4, 8.0 Hz, H-4), 6.76 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (100 MHz, MeOD) δ 173.6 (C═O), 165.7 (C═O), 153.2 (C), 146.2 (C), 143.6 (C), 135.3 (C), 131.9 (C), 131.8 (CH), 131.7 (CH), 130.7 (CH), 128.2 (CH), 125.2 (CH), 122.1 (CH), 121.1 (CH), 119.3 (CH), 115.3 (C); LRMS $[M - H]^-$ 333.40; HRMS calcd for $C_{16}H_{11}O_6ClNa$: *M*Na^+, 357.0142. Found: *M*Na^+, 357.0136.

3-(2-(4-Chlorophenyl)-1-carboxyvinyloxy)-2-hydroxybenzoic acid 30. Potassium hydroxide (0.50 mL) was added to a solution of diester **16** (14 mg, 0.04 mmol) in THF (0.50 mL). Water (0.25 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method B, affording the desired diacid **30** as a mixture of diastereomers as a colourless oil (11 mg, 86%, 83 : 17 *Z* : *E*). IR ν_{max} (ATR): 3446 (br), 2554, 1676, 1467, 1240 cm^{-1}; retention time: 32.67 min; *Z*: ^1H NMR (400 MHz, MeOD) δ 7.74 (2H, app. d, *J* 8.6 Hz, Ar-H), 7.55 (1H, dd, *J* 1.3, 8.0 Hz, H-6), 7.35 (1H, s, C═C*H*Ph), 7.34 (2H, app. d, *J* 8.9 Hz, Ar-H), 6.99 (1H, dd, *J* 1.3, 7.9 Hz, H-4), 6.75 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (100 MHz, MeOD) δ 173.6 (C═O), 165.9 (C═O), 153.2 (C), 146.1 (C), 142.5 (C), 136.4 (C), 132.8 (CH), 132.7 (2 × CH), 129.9 (2 × CH), 126.1 (CH), 125.1 (CH), 120.8 (CH), 119.3 (CH), 115.4 (C); *E*: ^1H NMR (400 MHz, MeOD) δ 7.69 (1H, dd, *J* 1.6, 8.1 Hz, Ar-H), 7.35–7.26 (5H, m, Ar-H + H-6 + H-4), 6.88 (1H, t, *J* 8.0 Hz, H-5), 6.37 (1H, s, C═C*H*Ph); ^{13}C NMR (100 MHz, MeOD) δ 132.8 (CH), 131.6 (2 × CH), 129.1 (2 × CH), 127.1 (CH), 126.2 (CH), 119.7 (CH), 119.7 (CH) 7 quaternary signals obscured; LRMS $[M - H]^-$ 349.53; HRMS calcd for $C_{16}H_{11}O_6ClNa$: *M*Na^+, 357.0142. Found: *M*Na^+, 357.0136.

3-(1-Carboxy-2-*m*-tolylvinyloxy)-2-hydroxybenzoic acid 31. Potassium hydroxide (0.9 mL) was added to a solution of diester **17** (29 mg, 0.08 mmol) in THF (1.0 mL). Water (0.45 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method A, affording the desired diacid **31** as a mixture of diastereomers as a colourless oil (19 mg, 76%, 63 : 37 *Z* : *E*). IR ν_{max} (ATR): 3043 (br), 2559, 1681, 1466, 1236, 755 cm^{-1}; retention time: 29.22 min; *Z*: ^1H NMR (400 MHz, MeOD) δ 7.56–7.51 (2H, m, Ar-H + H-6), 7.38 (1H, s, C═C*H*Ph), 7.20 (1H, t, *J* 7.6 Hz, Ar-H), 7.16–7.12 (2H, m, Ar-H), 6.94 (1H, dd, *J* 1.2, 8.0 Hz, H-4), 6.73 (1H, t, *J* 8.0 Hz, H-5), 2.28 (3H, s, Ar-*Me*); ^{13}C NMR (100 MHz, MeOD) δ 173.7 (C═O), 166.2 (C═O), 153.1 (C), 146.3 (C), 141.5 (C), 139.5 (C), 133.8 (CH), 132.0 (CH), 131.5 (CH), 130.6 (CH), 129.6 (CH), 128.5 (CH), 124.8 (CH), 120.4 (CH), 119.3 (CH), 115.1 (C), 21.4 (CH_3); *E*: ^1H NMR (400 MHz, MeOD) δ 7.66 (1H, dd, *J* 1.4, 8.0 Hz, Ar-H), 7.56–7.51 (2H, m, Ar-H + H-6), 7.24 (1H, dd, *J* 1.3, 7.9 Hz, H-4), 7.16–7.12 (1H, m, Ar-H), 7.06–7.05 (1H, m, Ar-H), 6.87 (1H, t, *J* 8.0 Hz, H-5), 6.43 (1H, s, C═C*H*Ph), 2.29 (3H, s, Ar-*Me*); ^{13}C NMR (100 MHz, MeOD) δ 173.5 (C═O), 166.3 (C═O), 154.4 (C), 145.4 (C), 145.3 (C), 138.7 (C), 134.7 (CH), 129.5 (CH), 128.9 (CH), 128.3 (CH), 127.1 (CH), 126.8 (CH), 125.6 (CH), 122.0 (CH), 119.6 (CH), 115.6 (C), 21.4 (CH_3); LRMS $[M + Na]^+$ 336.73; HRMS calcd for $C_{17}H_{14}O_6Na$: *M*Na^+, 337.0688. Found: *M*Na^+, 337.0691.

3-(1-Carboxy-2-*o*-tolylvinyloxy)-2-hydroxybenzoic acid 32. Potassium hydroxide (0.9 mL) was added to a solution of diester **18** (29 mg, 0.08 mmol) in THF (1.0 mL). Water (0.45 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method A, affording the desired diacid **32** as a mixture of diastereomers as a colourless oil (22 mg, 82%, 56 : 44 *Z* : *E*). IR ν_{max} (ATR): 3057 (br), 2538, 1678, 1463, 1227 cm^{-1}; retention time: 32.50 min; *Z*: ^1H NMR (400 MHz, MeOD) δ 7.84 (1H, app. d, *J* 7.7 Hz, Ar-H), 7.61 (1H, s, C═C*H*Ph), 7.50 (1H, dd, *J* 1.4, 8.0 Hz, H-6), 7.20–7.07 (3H, m, Ar-H), 6.97 (1H, dd, *J* 1.4, 8.0 Hz, H-4), 6.73 (1H, t, *J* 8.0 Hz, H-5), 2.41 (3H, s, Ar-*Me*); ^{13}C NMR (100 MHz, MeOD) δ 173.6 (C═O), 166.3 (C═O), 153.1 (C), 141.9 (C), 138.7 (C), 137.2 (C), 134.7 (CH), 132.3 (CH), 131.3 (CH),

130.4 (CH), 127.1 (CH), 126.4 (CH), 124.9 (CH), 120.7 (CH), 119.2 (CH), 115.1 (C), 20.2 (CH_3); *E*: ^{1}H NMR (400 MHz, MeOD) δ 7.69 (1H, dd, *J* 1.5, 8.0 Hz, Ar-H), 7.31 (1H, dd, *J* 1.5, 7.9 Hz, H-6), 7.20–7.07 (4H, m, Ar-H + H-4), 6.90 (1H, t, *J* 8.0 Hz, H-5), 6.45 (1H, s, C=C*H*Ph), 2.21 (3H, s, Ar-*Me*); ^{13}C NMR (100 MHz, MeOD) δ 173.5 (C=O), 165.9 (C=O), 154.6 (C), 146.5 (2 × C), 145.9 (C), 145.1 (CH), 130.4 (CH), 130.1 (CH), 128.7 (CH), 127.0 (CH), 126.2 (CH), 124.8 (CH), 121.0 (CH), 119.7 (CH), 115.7 (C), 20.2 (CH_3); LRMS $[M + Na]^+$ 336.87; HRMS calcd for $C_{17}H_{14}O_6Na$: *M*Na^+, 337.0688. Found: *M*Na^+, 337.0683.

3-(1-Carboxy-2-*p*-tolylvinyloxy)-2-hydroxybenzoic acid 33. Potassium hydroxide (1.1 mL) was added to a solution of diester **19** (37 mg, 0.11 mmol) in THF. Water (0.54 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked up according to Method A, affording the desired diacid **33** as a mixture of diastereomers as an oil (21 mg, 61%, 67 : 33 *Z* : *E*). IR ν_{max} (ATR): 3054 (br), 2564, 1679, 1467, 1237 cm^{-1}; *Z*: ^{1}H NMR (400 MHz, MeOD) δ 7.62 (2H, d, *J* 8.2 Hz, Ar-H), 7.53 (1H, dd, *J* 1.5, 8.0 Hz, H-6), 7.39 (1H, s, C=C*H*Ph), 7.15 (2H, d, *J* 8.2 Hz, Ar-H), 6.96 (1H, dd, *J* 1.5, 8.0 Hz, H-4), 6.73 (1H, t, *J* 8.0 Hz, H-5), 2.30 (3H, s, Ar-*Me*); ^{13}C NMR (100 MHz, MeOD) δ 173.6 (C=O), 166.3 (C=O), 153.1 (C), 146.3 (C), 141.4 (C), 140.9 (C), 131.4 (2 × CH), 131.1 (C), 130.4 (2 × CH), 128.3 (CH), 124.8 (CH), 120.3 (C), 119.3 (CH), 115.3 (C), 21.4 (CH_3); *E*: ^{1}H NMR (400 MHz, MeOD) δ 7.66 (1H, dd, *J* 1.6, 8.0 Hz, H-6), 7.26 (2H, d, *J* 8.0 Hz, Ar-H), 7.25 (1H, dd, *J* 1.6, 8.0 Hz, H-4), 7.10 (2H, d, *J* 8.0 Hz, Ar-H), 6.87 (1H, t, *J* 8.0 Hz, H-5), 6.46 (1H, s, C=C*H*Ph), 2.30 (3H, s, Ar-*Me*); ^{13}C NMR (100 MHz, MeOD) δ 173.5 (C=O), 166.3 (C=O), 154.3 (C), 145.6 (C), 144.9 (C), 139.0 (C), 131.8 (C), 130.1 (2 × CH), 129.7 (2 × CH), 126.6 (CH), 125.4 (CH), 122.5 (CH), 119.6 (CH), 115.7 (C), 21.3 (CH_3); LRMS $[M + Na]^+$ 336.73; HRMS calcd for $C_{17}H_{14}O_6Na$: *M*Na^+, 337.0688. Found: *M*Na^+, 337.0681.

3-(1-Carboxy-2-(3-hydroxyphenyl)vinyloxy)-2-hydroxybenzoic acid 34. Potassium hydroxide (0.31 mL) was added to a solution of diester **20** (12 mg, 0.03 mmol) in THF. Water (0.69 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method B, affording the desired diacid **34** as a mixture of diastereomers as a white solid (5.5 mg, 56%, 63 : 37 *Z* : *E*). IR ν_{max} (ATR): 3199 (br), 2575, 1687, 1469, 1239 cm^{-1}; retention time: 25.58 min; mp 120–122. *Z*: ^{1}H NMR (400 MHz, MeOD) δ 7.54 (1H, dd, *J* 1.5, 8.0 Hz, H-6), 7.31 (1H, s, C=C*H*Ph), 7.26–7.22 (1H, m, Ar-H), 7.21–7.17 (1H, m, Ar-H), 7.16 (1H, t, *J* 7.8 Hz, Ar-H), 6.96 (1H, dd, *J* 1.5, 8.0 Hz, H-4), 6.78–6.75 (1H, m, Ar-H), 6.74 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (100 MHz, MeOD) δ 174.0 (C=O), 166.3 (C=O), 158.7 (C), 153.2 (C), 146.3 (C), 141.9 (C), 135.2 (C), 130.6 (CH), 127.9 (CH), 125.0 (CH), 123.0 (CH), 120.3 (CH), 119.0 (CH), 117.9 (CH), 117.7 (CH), 115.7 (C); *E*: ^{1}H NMR (400 MHz, MeOD) δ 7.68 (1H, dd, *J* 1.6, 8.0 Hz, H-6), 7.26–7.22 (1H, m, H-4), 7.09 (1H, t, *J* 8.0 Hz, Ar-H), 6.85 (1H, t, *J* 8.0 Hz, H-5), 6.83–6.77 (2H, m, Ar-H), 6.67 (1H, dd, *J* 2.2, 8.0 Hz, Ar-H), 6.30 (1H, s, C=C*H*Ph); ^{13}C NMR (100 MHz, MeOD) δ 173.8 (C=O), 166.8 (C=O), 158.2 (C), 154.5 (C), 146.6 (C), 145.2 (C), 136.3 (C), 130.0 (CH), 127.0 (CH), 125.5 (CH), 121.4 (CH), 120.1 (CH), 119.3 (CH), 116.6 (CH), 116.3 (C) 1 signal obscured; LRMS $[M - H]^-$ 314.87; HRMS calcd for $C_{16}H_{12}O_7Na$: *M*Na^+, 339.0481. Found: *M*Na^+, 339.0472.

3-(1-Carboxy-2-(2-hydroxyphenyl)vinyloxy)-2-hydroxybenzoic acid 35. Potassium hydroxide (0.24 mL) was added to a solution of *Z*-rich diester **21** (9 mg, 0.02 mmol) in THF. Water (0.76 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method B, affording the desired pure *Z* diacid **35** as a white solid (3 mg, 38%). IR ν_{max} (ATR): 3257 (br), 2566, 1679, 1459, 1244, 754 cm^{-1}; retention time: 26.63 min; mp 137–139 °C; ^{1}H NMR (400 MHz, MeOD) δ 7.92 (1H, dd, *J* 1.6, 7.9 Hz, Ar-H), 7.90 (1H, s, C=C*H*Ph), 7.52 (1H, dd, *J* 1.5, 8.0 Hz, H-6), 7.13 (1H, ddd, *J* 1.6, 7.5, 8.2 Hz, Ar-H), 6.92 (1H, dd, *J* 1.5, 8.0 Hz, H-4), 6.82 (1H, dd, *J* 0.8, 8.2 Hz, Ar-H), 6.73 (1H, ddd, *J* 0.8, 7.5, 7.9 Hz, Ar-H), 6.70 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (100 MHz, MeOD) δ 174.3 (C=O), 166.9 (C=O), 157.4 (C), 153.0 (C), 146.4 (C), 140.8 (C), 132.0 (CH), 131.3 (CH), 124.7 (CH), 122.4 (CH), 121.0 (C), 120.7 (CH), 119.5 (CH), 118.8 (CH), 117.1 (C), 116.3 (CH); LRMS $[M - H]^-$ 314.87; HRMS calcd for $C_{16}H_{12}O_7Na$: *M*Na^+, 339.0481. Found: *M*Na^+, 339.0474.

3-(1-Carboxy-2-(4-hydroxyphenyl)vinyloxy)-2-hydroxybenzoic acid 36. Potassium hydroxide (0.69 mL) was added to a solution of diester **22** (27 mg, 0.07 mmol) in THF. Water (0.34 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method B, affording the desired pure *Z* diacid **36** as a colourless oil (13 mg, 59%). IR ν_{max} (ATR): 3110 (br), 2562, 1690, 1604, 1466, 1242 cm^{-1}; retention time: 26.00 min; ^{1}H NMR (400 MHz, MeOD) δ 7.61 (2H, d, *J* 8.8 Hz, Ar-H), 7.52 (1H, dd, *J* 1.5, 8.0 Hz, H-6), 7.36 (1H, s, C=C*H*Ph), 6.90 (1H, dd, *J* 1.5, 8.0 Hz, H-4), 6.74 (2H, d, *J* 8.8 Hz, Ar-H), 6.69 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (100 MHz, MeOD) δ 174.4 (C=O), 167.0 (C=O), 160.4 (C), 152.9 (C), 146.3 (C), 139.4 (C), 133.4 (2 × CH), 128.6 (CH), 125.4 (C), 124.7 (CH), 119.1 (CH), 118.7 (CH), 117.4 (C), 116.6 (2 × CH); LRMS $[M - H]^-$ 314.93; HRMS calcd for $C_{16}H_{12}O_7Na$: *M*Na^+, 339.0481. Found: *M*Na^+, 339.0473.

3-(2-(3-Trifluoromethylphenyl)-1-carboxyvinyloxy)-2-hydroxybenzoic acid 37. Potassium hydroxide (0.75 mL) was added to a solution of diester **23** (25 mg, 0.06 mmol) in THF (0.75 mL). Water (0.40 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method B, affording the desired diacid **37** as a mixture of diastereomers as a colourless oil (19 mg, 80%, 77 : 23 *Z* : *E*). IR ν_{max} (ATR): 3072 (br), 2542, 1666, 1465, 1329, 1120 cm^{-1}; retention time: 34.11 min; *Z*: ^{1}H NMR (500 MHz, MeOD) δ 8.07 (1H, s, C=C*H*Ph), 7.95 (1H, app. d, *J* = 7.8 Hz, Ar-H), 7.56–7.51 (3H, m, Ar-H + H-6), 7.41 (1H, app. s, Ar-H), 7.00 (1H, dd, *J* 1.4, 8.0 Hz, H-4), 6.75 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (125 MHz, MeOD) δ 173.6 (C=O), 165.8 (C=O), 153.2 (C), 146.0 (C), 143.8 (C), 132.1 (C, q, $^2J_{C-F}$ 32 Hz), 130.5 (CH), 129.7 (CH), 127.6 (CH, q, $^3J_{C-F}$ 4.0 Hz), 126.7 (CH, q, $^3J_{C-F}$ 3.7 Hz), 125.3 (CH), 125.2 (CH), 125.4 (C, q, $^1J_{C-F}$ 272 Hz), 121.0 (CH), 119.2 (CH), 115.6 (C) 1 signal obscured; *E*: ^{1}H NMR (400 MHz, MeOD) δ 7.70 (1H, dd, *J* 1.4, 8.0 Hz, Ar-H), 7.64 (1H, s, Ar-H), 7.60 (1H, app. d, *J* 7.9 Hz, H-6), 7.56–7.51 (1H, m, Ar-H), 7.46 (1H, t, *J* 7.8 Hz, Ar-H), 7.29 (1H, dd, *J* 1.4, 8.0

Hz, H-4), 6.89 (1H, t, *J* 8.0 Hz, H-5), 6.39 (1H, s, C=C*H*Ph); ^{13}C NMR (125 MHz, MeOD) δ 173.5 (C=O), 165.8 (C=O), 154.6 (C), 147.7 (C), 144.7 (C), 132.2 (CH), 132.0 (CH), 131.4 (C, q, $^{2}J_{C-F}$ 36 Hz), 127.4 (CH), 126.6 (CH, q, $^{3}J_{C-F}$ 4.0 Hz), 126.5 (CH), 125.0 (CH, q, $^{3}J_{C-F}$ 3.7 Hz), 125.7 (C, q, $^{1}J_{C-F}$ 272 Hz), 119.7 (CH), 118.2 (CH), 116.3 (C) 1 signal obscured; LRMS $[M + H]^+$ 367.47; HRMS calcd for $C_{17}H_{11}F_3O_6Na$: *M*Na$^+$, 391.0405. Found: *M*Na$^+$, 391.0400.

3-(2-(2-Trifluoromethylphenyl)-1-carboxyvinyloxy)-2-hydroxybenzoic acid 38. Potassium hydroxide (0.65 mL) was added to a solution of pure *Z* diester **24** (22 mg, 0.05 mmol) in THF (0.65 mL). Water (0.40 mL) was then added and the reaction stirred at rt for 48 h. The mixture was worked-up according to Method B, affording the desired pure *Z* diacid **38** as a colourless oil (16 mg, 75%). IR ν_{max} (ATR): 3439 (br), 2517, 1647, 1466, 1314, 1113 cm^{-1}; retention time: 32.54 min; ^{1}H NMR (500 MHz, MeOD) δ 8.06 (1H, app. d, *J* = 8.0 Hz, Ar-H), 7.71 (1H, app. d, *J* 7.8 Hz, Ar-H), 7.60 (1H, s, C=C*H*Ph), 7.56–7.51 (2H, m, Ar-H + H-6), 7.45 (1H, t, *J* 8.0 Hz, Ar-H), 6.96 (1H, app. d, *J* 8.0 Hz, H-4), 6.73 (1H, t, *J* 8.0 Hz, H-5); ^{13}C NMR (125 MHz, MeOD) δ 173.7 (C=O), 165.7 (C=O), 153.2 (C), 146.3 (C), 144.6 (C), 133.1 (CH), 132.3 (CH), 130.0 (CH), 129.4 (C, q, $^{2}J_{C-F}$ 30 Hz), 126.8 (2 × CH, q, $^{3}J_{C-F}$ 5.8 Hz), 125.3 (CH), 125.6 (C, q, $^{1}J_{C-F}$ 276 Hz), 121.3 (CH), 119.1 (CH), 116.1 (C) 1 signal obscured; LRMS $[M + H]^+$ 367.53; HRMS calcd for $C_{17}H_{11}F_3O_6Na$: *M*Na$^+$, 391.0405. Found: *M*Na$^+$, 391.0400.

Enzyme inhibition assays

Over-expression and purification of MbtI was achieved using the method previously reported.[16]

Inhibition constants against MbtI were measured by activity assays in which the production of pyruvate from chorismate by each enzyme was coupled to the oxidation of NADH using lactate dehydrogenase (LDH). Loss of NADH was followed at a UV absorbance of 340 nm. Assays were performed on a Cary 4000 UV spectrophotometer (Varian) equipped with a 6 × 6 multi-cell holder using 1 cm pathlength quartz cuvettes and temperature control (25 °C). MbtI activity assays consisted of 50 mM Tris·HCl (pH 8), 5 mM $MgCl_2$, 0.25 mM NADH, 2 mg mL^{-1} LDH, 40 mg mL^{-1} MbtI, and 1–64 mM chorismate.

Inhibitors were dissolved in water or DMSO depending on solubility and added to assays at concentrations ranging from 50 nM to 2 mM. Assays were pre-incubated with inhibitor present at 25 °C for 10 min and were initiated by the addition of chorismate. Reaction rates were measured by least-squares fitting of the initial decrease in absorbance with time. Kinetic parameters were determined by nonlinear fitting of reaction rates to a competitive model of inhibition using GraphPad Prism software version 5.02 for Windows (GraphPad Software Inc.).

Whole cell inhibition assays

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 inhibitors against *M. tuberculosis* growth were measured by a resazurin reduction microplate assay, using the procedure previously described by Taneja and Tyagi.[22] *M. tuberculosis* grown to mid-exponential phase 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 × 10^4 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 instrument 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.

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