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Design and Synthesis of Aryl Ether Inhibitors of the *Bacillus Anthracis* Enoyl-ACP Reductase

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The problem of increasing bacterial resistance to the current generation of antibiotics is well documented. Known resistant pathogens such as methicillin-resistant Staphylococcus aureus *are becoming more prevalent, while the potential exists for developing drug-resistant pathogens for use as bioweapons, such as* Bacillus anthracis*. The biphenyl ether antibacterial agent, triclosan, exhibits its broad-spectrum activity by targeting the fatty acid biosynthetic pathway through inhibition of enoyl-acyl carrier protein reductase (ENR) and provides a potential scaffold for the development of new, broad-spectrum antibiotics. We used a structure-based approach to develop novel aryl ether analogues of triclosan that target ENR, the product of the* fabI *gene, from* B. anthracis *(*Ba*ENR). Structure-based design methods were used for the expansion of the compound series including X-ray crystal structure determination, molecular docking, and QSAR methods. Structural modifications were made to both phenyl rings of the 2-phenoxyphenyl core. A number of compounds exhibited improved potency against* Ba*ENR and increased efficacy against both the Sterne strain of* B. anthracis *and the methicillin-resistant strain of* S. aureus*. X-ray crystal structures of* Ba*ENR in complex with triclosan and two other compounds help explain the improved efficacy of the new compounds and suggest future rounds of optimization that might be used to improve their potency.*

Introduction

The increasing prevalence of antibiotic-resistant bacteria is well documented. The 2004 monograph by the Infectious Diseases Society of America noted that the incidence of methicillin-resistant *S. aureus* (MRSA) in particular, has increased quite rapidly over the last two decades.[1] Recent summaries have documented that *Staphylococci* are among the most common causes of nosocomial infections, and that resistance to β-lactams and glycopeptides is complicating treatment of those infections.[2] Particularly alarming is a recent JAMA article and accompanying editorial, which noted that deaths from invasive MRSA in 2005 were comparable to, or exceeded those from HIV/AIDS.[3,4] This rapid increase in bacterial resistance to current antibiotics is a strong motivation for the development of new antibacterials with alternate modes of action.

Drug resistance in pathogens that might be used as bioweapons is also of concern. Natural isolates of *B. anthracis* have been reported to show resistance to some antibiotics such as penicillin G, amoxicillin, erythromycin, cefuroxime, sulfamethoxazole, trimethoprim, cefotaxime-sodium, aztreonam, deftazidime[5–8] and ofloxacin,[9] as well as tetracycline and penicillin.[10] Furthermore, two reports described the potential to develop resistant *B. anthracis* strains through standard microbial selection procedures.[11,12] While previous acts of bioterrorism used a *B. anthracis* strain that was susceptible to conventional antibiotics, future incidents may involve more virulent *B. anthracis* strains resistant to conventional antibiotics. Because anthrax has been rated first or second for potential bioterrorism impact, comparable to smallpox,[13,14] and may be readily adapted to biowarfare applications,[14] there is an incentive for the development of antibiotics with novel modes of action that could be used to combat drug-resistant bacteria.[15]

Validated targets for antibiotic development are the fatty acid biosynthesis pathways essential for bacterial growth.[16,17] Fatty acids are synthesized by mammals (FAS I) and bacteria (FAS II) by substantially different biosynthetic mechanisms, enabling bacteria-specific drug targeting. FAS I involves a single multifunctional enzyme-acyl carrier protein (ACP) complex,

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whereas FAS II incorporates several small monofunctional enzymes that operate in conjunction with ACP-associated substrates.[18] Recent studies have revealed that the genes responsible for FAS II are essential in *Bacillus subtilis*, a close relative of *B. anthracis*.[19]

Enoyl-ACP reductase (ENR), the product of the *fabI* gene in *B. anthracis* (*Ba*ENR), is a key NADH-dependent enzyme in FAS II that catalyzes the final and rate-determining step of chain elongation.[20] Research has shown that enoyl-ACP reductase is efficiently inhibited in some pathogens by antibacterial agents including isoniazid,[21] diazaboranes,[22–24] triclosan,[25–28] and several other small-molecule inhibitors.[29–35] API-1252, a recently developed FabI inhibitor, shows excellent in vitro activity against clinical isolates of *Staphylococcus epidermidis* and *S. aureus*.[36a] Another novel FabI inhibitor, CG400462, was recently reported to show efficacy against *S. aureus* infected mice.[36b] These studies clearly indicate that inhibition of enoyl-ACP reductase is a viable approach to develop new antibacterials with novel modes of action.

Triclosan, a 2-phenoxyphenol, is a well-known, broad-spectrum antibacterial that is used in a number of consumer products, such as toothpastes, soaps and plastics. It has been shown to inhibit the growth of *Escherichia coli*,[26,37] *Pseudomonas aeruginosa*,[38] and *S. aureus*.[32] Originally, triclosan was thought to be a nonspecific antibacterial acting against bacterial cell membranes, however, the mode of action was later shown to be inhibition of bacterial fatty acid synthesis at the enoyl-acyl carrier protein reductase step.[39,40] Triclosan inhibits ENR, the gene product of *fabI*, *fabL*, and *inhA* in a number of microorganisms, leading to several attempts to develop new triclosan-derived antibacterials.[26,41–45] Further investigation into the broad-spectrum activity of triclosan derivatives has been deterred by the variability in effectiveness against different species; the IC_{50} values range from 70 and 73 nM respectively in *S. aureus*[32] and *Plasmodium falciparum* (*Pf*ENR)[42] to only 7.25 μM in *E. coli*.[26,37] Due to this large range in activity, it would be valuable to explore the inhibitory action of additional triclosan-like aryl ether analogues against individual organisms to maximize the specificity. Initially focusing on *B. anthracis*, we determined the IC_{50} value of triclosan against *Ba*ENR to be 0.6 μM with a minimum inhibitory concentration (MIC) of 3.1 $\mu g\,mL^{-1}$.[46] Herein we describe our approaches to improve its efficacy through structural modifications to the 2-phenoxyphenol core using a structure-based design approach that relies on the crystal structure of *Ba*ENR with triclosan bound in the active site.[46] Additional *Ba*ENR crystal structures involving newly designed diphenyl ethers are also presented and discussed. We found that at least two compounds exhibit improved activity against both the ΔANR, and Sterne strain of *B. anthracis*, as well as MRSA.[1]

[1] *The ΔANR strain, containing neither pXO1 (toxin) nor pXO2 (capsule) plasmids, was used for enzymatic assays. The Sterne strain, containing only the pXO1 (toxin) plasmid, was used in antibacterial testing.*

Results and Discussion

Synthesis of the inhibitors

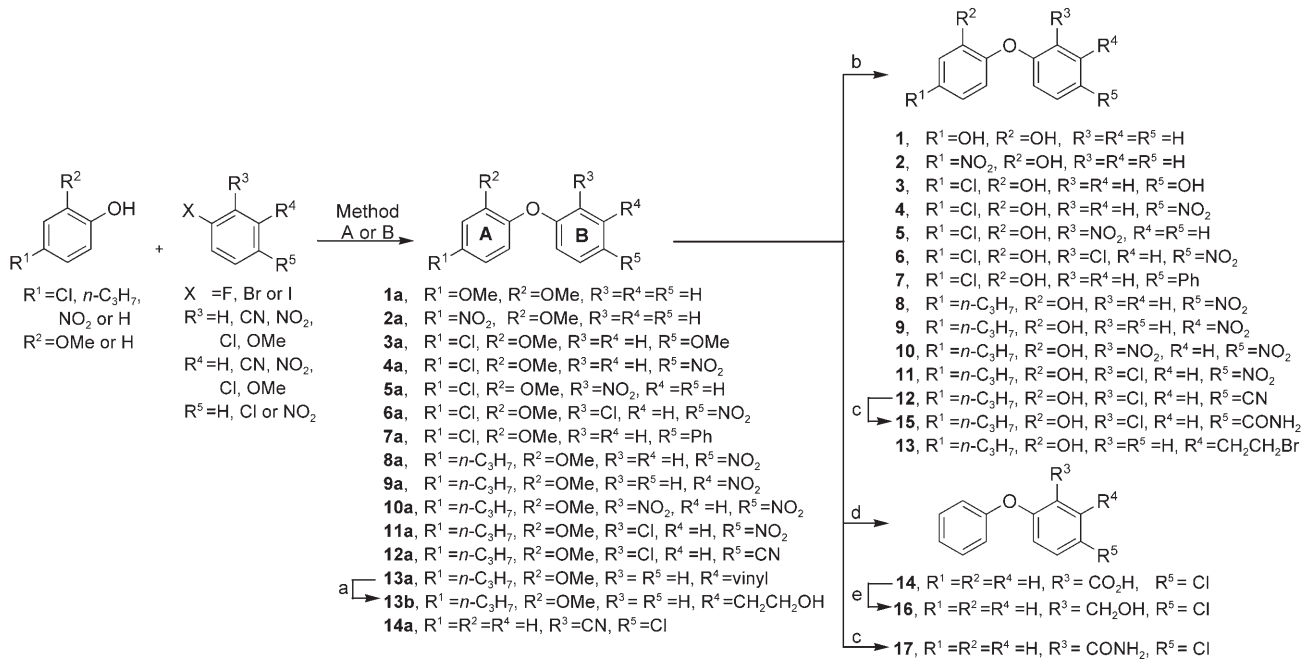
The 2-phenoxyphenol core was prepared from the corresponding methoxy derivatives, synthesized from commercially available materials by nucleophilic aromatic substitution reaction (Method A) or Cu-catalyzed coupling reaction (Methods B and C)[47] followed by demethylation (Method D) (Scheme 1).

Method A involves the reaction of an appropriate phenol with a fluoro-aromatic compound in the presence of K_2CO_3 and was used to prepare a variety of 2-phenoxyphenol derivatives bearing an electron-withdrawing group on ring B, namely NO_2, or CN groups (**4**–**6**, **8**–**12**, and **14**). Compound **14** was synthesized by base-catalyzed hydrolysis of the benzonitrile **14a** in EtOH at reflux.[48] Subsequent reduction of the carboxylic acid using $NaBH_4$ in the presence of $BF_3{\cdot}Et_2O$ gave benzylic alcohol **16**.[49] Attempts to demethylate **13b** using excess BBr_3 resulted in the formation of brominated analogue **13** as a major product. Carboxamides **15** and **17** were prepared from the corresponding benzonitriles by base-catalyzed hydrolysis in the presence of hydrogen peroxide.[48] Anilines **20** and **21** were synthesized by catalytic hydrogenation followed by demethylation of nitro intermediates **4a** and **8a**, respectively. The aniline intermediates **19a** and **20a** were acetylated and subsequently demethylated to give acetamides **19** and **18**. Similarly, tosylation followed by demethylation under standard conditions gave sulfonamide **22** from aniline **20a** (Scheme 2).

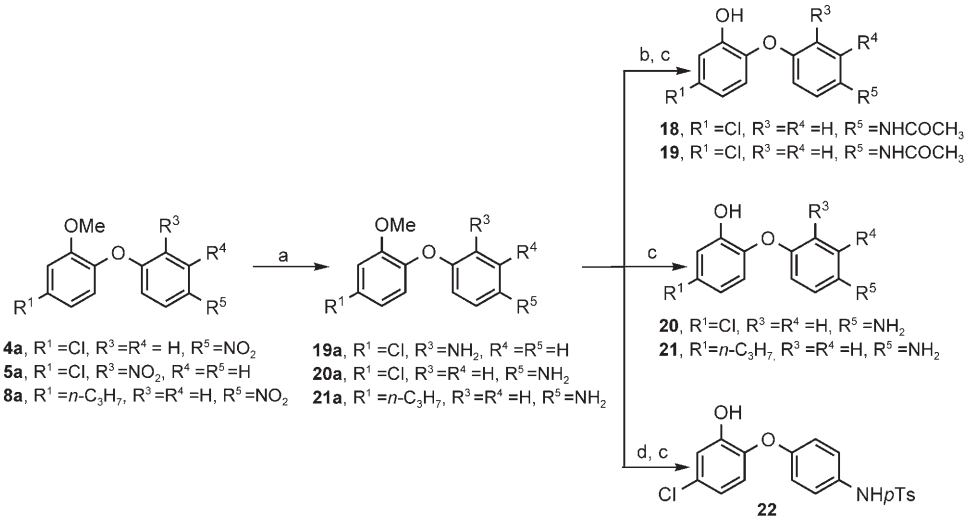
Method B involves the Cu-catalyzed coupling reaction of an appropriate phenol with a variety of aromatic halides under thermal conditions. This method is versatile, and was used to synthesize electron-rich diphenyl ethers **1**, **3**, and **7** (Scheme 1). Heteroaromatic B ring analogues (**23**–**25**) were prepared similarly (Scheme 3).

Method C is a mild copper-promoted C-O coupling reaction between arylboronic acids and phenols that was employed to synthesize a variety of aryl ethers (Schemes 4–7). This coupling reaction complements nucleophilic aromatic substitutions (Method A) and Ullman-type couplings (Method B) described above for the synthesis of aryl ethers. Although this reaction worked well with *meta*- and *para*-substituted phenylboronic acids, coupling of 2-methoxyphenols with *ortho*-substituted phenylboronic acids, as well as with heteroaromatic boronic acids failed, presumably due to unfavorable steric interactions.

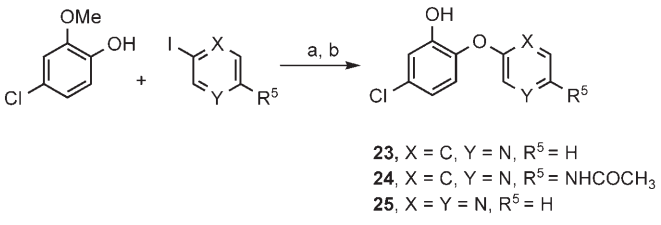
In Scheme 4, coupling of phenylboronic acid with commercially available 4-allyl-2-methoxyphenol and 4-hydroxy-3-methoxybenzaldehyde gave diphenyl ether intermediates **27a** and **28a**, respectively, in high yields. Allyl and aldehyde functional groups were tolerated under these mild reaction conditions. The allyl group of **27a** was further functionalized to the 1,2-diol **26** via dihydroxylation and demethylation, or the *n*-propyl-substituted diphenyl ether **27** via catalytic hydrogenation and demethylation. Similarly, the aldehyde **28a** was either reduced to an alcohol and demethylated (to give **28**), or transformed into a series of alkyl amines by reductive amination to give compounds **29**–**31** after demethylation.



Scheme 1. Synthesis of compounds **1**–**17**: When X = F and $R^3 = R^4 = H$, NO_2, CN, Cl, Method A: K_2CO_3, DMSO, 100 °C, 8–12 h. When X = Br or I and $R^3 = R^4 = H$, OMe, Ph, Method B: KO*t*Bu, DMF, $(CuOTf)_2$·PhH, 140 °C, 16–20 h. a) BH_3·THF, 3 M NaOH, H_2O_2, RT, 4–6 h; b) Excess BBr_3, CH_2Cl_2, −78 °C to RT, 2–6 h; c) 35% H_2O_2, 3 N NaOH, EtOH, 30 °C, 18 h; d) **14a**, 25% NaOH, EtOH, reflux, 20 h; e) $NaBH_4$, BF_3·Et_2O, THF, RT to reflux, 1 h.



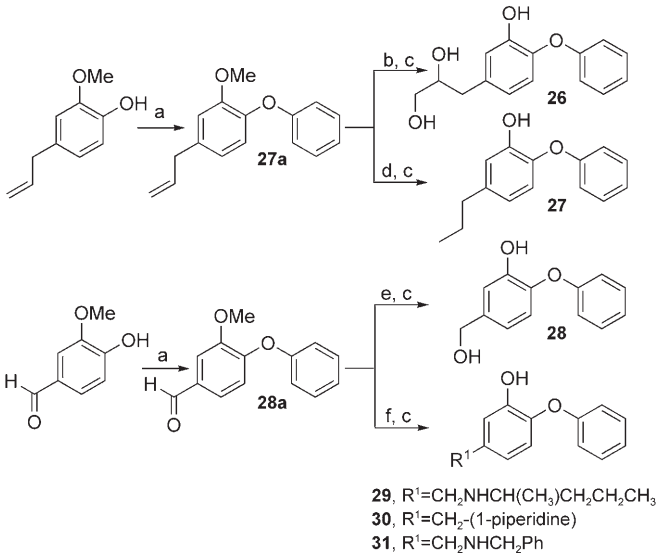
Scheme 2. Synthesis of compounds **18**–**22**: a) Pd/C, H_2, EtOH, RT, 2–6 h; b) Ac_2O, DMAP, Et_3N, CH_2Cl_2, RT, 3–6 h; c) Excess BBr_3, CH_2Cl_2, −78 °C to RT, 2–6 h; d) 4-toluenesulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C to RT, 3 h.



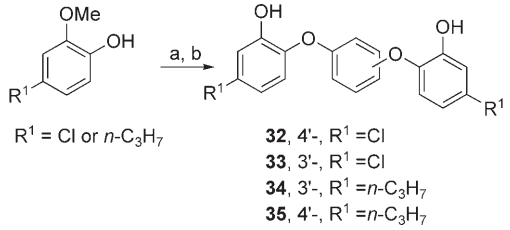
Scheme 3. Synthesis of compounds **23**–**25**: a) Method B: KO*t*Bu, DMF, $(CuOTf)_2$·PhH, 140 °C, 14–18 h; b) Excess BBr_3, CH_2Cl_2, −78 °C to RT, 2–6 h.

Scheme 5 illustrates the coupling of 2-methoxy-4-chlorophenol or 2-methoxy-4-propylphenol with commercially available *meta*- and *para*-substituted phenyldiboronic acids to give the triphenyl ethers **32**–**35** in two steps. While the coupling of *meta*-substituted phenyldiboronic acid and phenols resulted in high yields, *para*-substituted phenyldiboronic acid did not react well (less than 10% yield).

Schemes 6 and 7 illustrate the synthetic routes to *meta*- and *para*-substituted ring B diphenyl ether analogues from commercially available arylboronic acids bearing both electron-donating and electron-withdrawing functional groups. These functional groups were further elaborated to obtain diverse substitutions on ring B. Benzoic acid **39** was obtained by hydrolysis of the corresponding methyl benzoate **37** while compound **42** was obtained by the reduction of the ketone **40** using $NaBH_4$. Oxidation of the methylthioether group in **41a** afforded the methyl sulfoxide **43** and methyl sulfone **44**, respectively, after demethylation. Coupling 4-allyl-2-methoxyphenol with the corresponding arylboronic acids gave allylic intermediates **45a** and **46a**; reduction of the allylic side



Scheme 4. Synthesis of compounds **26**–**31**: a) Phenylboronic acid, $Cu(OAc)_2$, Et_3N, CH_2Cl_2, air, RT, 16 h; b) OsO_4, NMO, THF, RT, overnight; c) BBr_3, CH_2Cl_2, −78 °C, 2 h; d) H_2, Pd/C, EtOAc, RT, 2 h; e) $NaBH_4$, MeOH, 0 °C, 1 h; f) amine, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, RT, overnight.



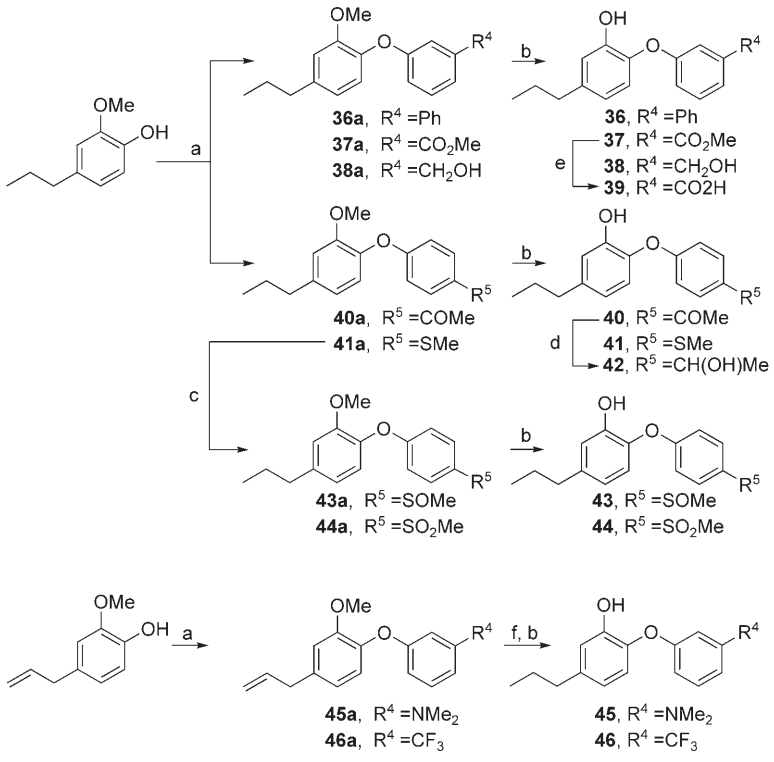
Scheme 5. Synthesis of compounds **32**–**35**: a) $Cu(OAc)_2$, *meta*- or *para*-phenyldiboronic acid, Et_3N, CH_2Cl_2, air, RT, 16 h; b) BBr_3, CH_2Cl_2, −78 °C, 2 h.

chain via catalytic hydrogenation, and subsequent demethylation gave compounds **45** and **46**.

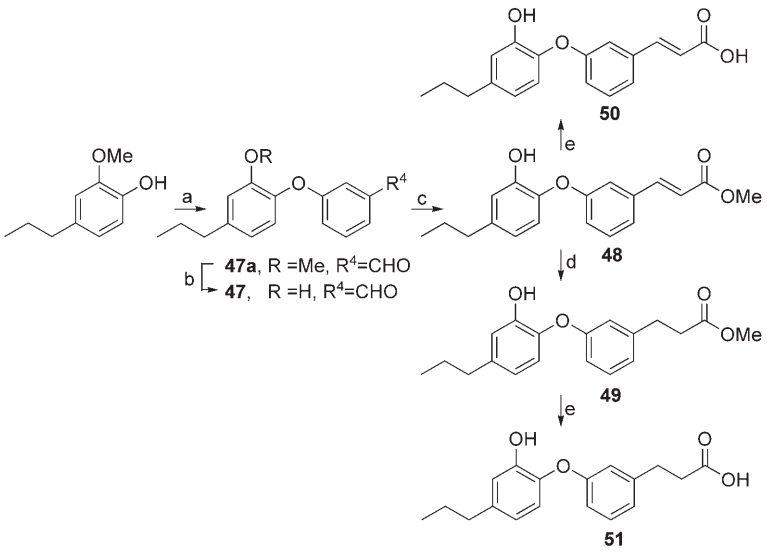
In Scheme 7, 2-methoxy-4-propylphenol was coupled with 3-formylphenylboronic acid to give the aldehyde **47a**; subsequent demethylation gave compound **47**. The *meta*-aldehyde group in **47** underwent a Wittig reaction, and two-carbon elongated side chain analogues **48**–**51** were obtained by following similar hydrogenation and hydrolysis protocols.

Evaluation of the active site

Initial assay results indicate that triclosan is an effective inhibitor of *Ba*ENR, and has good antibacterial activity against *B. anthracis* (Table 1). We conducted structure-activity relationship studies on a number of aryl ether derivatives to improve the potency of this lead compound, with the goal of optimizing the activity against *Ba*ENR. The first structural modifications were directed at ring A of triclosan. As expected, the hydrogen bonding interaction of the phenolic hydroxy group on ring A is critical for *Ba*ENR inhibitory activity. Conversion of the phenol group to an ether resulted in complete loss of activity (compounds **52** and **53**), while substitution by an amino functionality (compound **54**), carboxylic acid (compound **14**), or a carboxamide (compound **17**) resulted in no inhibition at 1 μM. Replacing the phenol with benzylic alcohol (compound **16**) gave no improvement in activity. We recently reported the X-ray structure of triclosan bound to *Ba*ENR,[46] which helps explain this lack of activity. The binding geometry of triclosan, shown in Figure 1a, is similar to that seen with analogous proteins from other organisms. The phenolic "ring A" of triclosan π stacks with the nicotinamide ring of NAD^+ while the hydroxy group is involved in hydrogen bonds with the phenol side chain of Tyr 157 and the 2′-hydroxy group of nicotinamide ribose. Removal of these hydrogen bonds by substitution at the 2-position would have a substantial impact on the binding energy of these compounds, confirmed by the experimental results (Table 1). The diphenyl ether linkage is also within hydrogen bonding distance to the 2′-hydroxy group of nicotinamide ribose, thereby adding increased binding energy between *Ba*ENR and the NAD^+-triclosan complex. Based on these findings, our design efforts focused on 2-phenoxyphenol as the key scaffold, and on optimizing the substitution of rings A and B to maximize the van der Waals, electrostatic, and hydrogen bonding interactions in the active site. The design of compounds in this investigation was also based on comparison of calculated molecular properties (ClogP, ALOGpS and TPSA) with those of triclosan.[50]



Scheme 6. Synthesis of compounds **36**–**46**: a) $Cu(OAc)_2$, substituted phenylboronic acids, Et_3N, CH_2Cl_2, air, RT, 16 h; b) BBr_3, CH_2Cl_2, −78 °C to RT, 2 h; c) *m*-CPBA, CH_2Cl_2, 0 °C, 10 min; d) $NaBH_4$, MeOH, 0 °C, 1 h; e) $LiOH{\cdot}H_2O$, MeOH, H_2O, RT, 2 h; f) H_2, Pd/C, EtOAc, RT, 2 h.



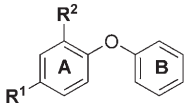
Scheme 7. Synthesis of compounds **47**–**51**: a) $Cu(OAc)_2$, 3-formylphenylboronic acid, Et_3N, CH_2Cl_2, air, RT, 16 h; b) BBr_3, CH_2Cl_2, −78 °C to RT, 2 h; c) $Ph_3P{=}CHCO_2Me$, THF, reflux, overnight; d) H_2, Pd/C, EtOAc, RT, 2 h; e) $LiOH{\cdot}H_2O$, MeOH, H_2O, RT, 2 h.

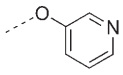
Connolly surface maps with various properties were generated for the active site of *Ba*ENR; Figure 2a maps the charge distribution to the surface, Figure 2b maps the lipophilicity, and Figure 2c maps the hydrogen bonding opportunities found on the active site surface. Full evaluation and consideration of these active site surface maps contribute significantly to the structure-based design approach. For this system, the shape of the active site indicates that the R^1 position on ring A and the R^4 and/or R^5 positions on ring B are suitable sites for introducing bulky substituents. The ring A pocket appears to be neutral in charge (Figure 2a), lacking in hydrogen bonding opportunities (Figure 2c), and highly hydrophobic (Figure 2b); for these reasons, we designed inhibitors with increased lipophilic R^1 substituents on ring A to maximize the hydrophobic interactions in this pocket, and minimize steric constraints.

Substitutions on ring A

Compound **55**, bereft of substituents on ring B, was previously shown to be a potent inhibitor of *E. coli* ENR.[37] Compound **55**, used as a reference in this study, shows similar activity to that of triclosan against *Ba*ENR, although its MIC value against ΔANR *B. anthracis* is an order of magnitude lower (Table 1). Replacement of the R^1 chloro substituent with polar, hydrophilic functionalities, such as OH (compound **1**) and NO_2 (compound **2**), decreased the inhibitory activity against *Ba*ENR. Although compound **2** has fair antibacterial activity (MIC = 5.8 μg mL^{-1}), its poor enzyme inhibitory activity suggests a different mechanism of action. Poor solubility of these diphenyl ether derivatives made the introduction of hydrophilic groups at the R^1 position necessary (in **26**, **28**, and **29**--**31**), which resulted in decreased inhibitory activity (Table 1). This is in agreement with the surface-map property analysis of the active site from the *Ba*ENR–triclosan X-ray structure (Figure 2a–c). The surface maps show a hydrophobic pocket, approximately 6 Å in diameter, near the R^1 position of ring A. Moreover, Figure 2c shows there are no opportunities for additional hydrogen bonds to

Table 1. *Ba*ENR inhibition and antibacterial activity for modifications of ring A.



Compound	R^1	R^2	IC_{50} [μM] or % inhibition at 1 μM	MIC[b] [μg mL^{-1}]
Triclosan			0.6 ± 0.0	3.1
52	Cl		0.5%	NT
53	Cl	OMe	0%	NT
54	Cl	NH_2	4.4%	NT
14	Cl	CO_2H	0%	NT
16	Cl	CH_2OH	1.8%	NT
17	Cl	$CONH_2$	0%	NT
55	Cl	OH	0.5 ± 0.1	32
1	OH	OH	6.3 ± 0.4	64
2	NO_2	OH	> 50	5.8
26	$CH_2CH(OH)CH_2OH$	OH	9.8%	> 104
28	CH_2OH	OH	5.0%	43
29	$CH_2NHCH(CH_3)CH_2CH_2CH_3$	OH	0%	> 109
30	CH_2-(1-piperidine)	OH	0%	> 113
31	CH_2NHCH_2Ph	OH	7.9%	> 122
27	*n*-propyl	OH	> 0.8[a]	22.8

[a] Saturation with inhibitor was not obtained over the concentration range tested. The percent inhibition of *Ba*ENR showed a linear response to increasing inhibitor concentrations. [b] MIC values are against ΔANR *B. anthracis*. NT = Not tested.

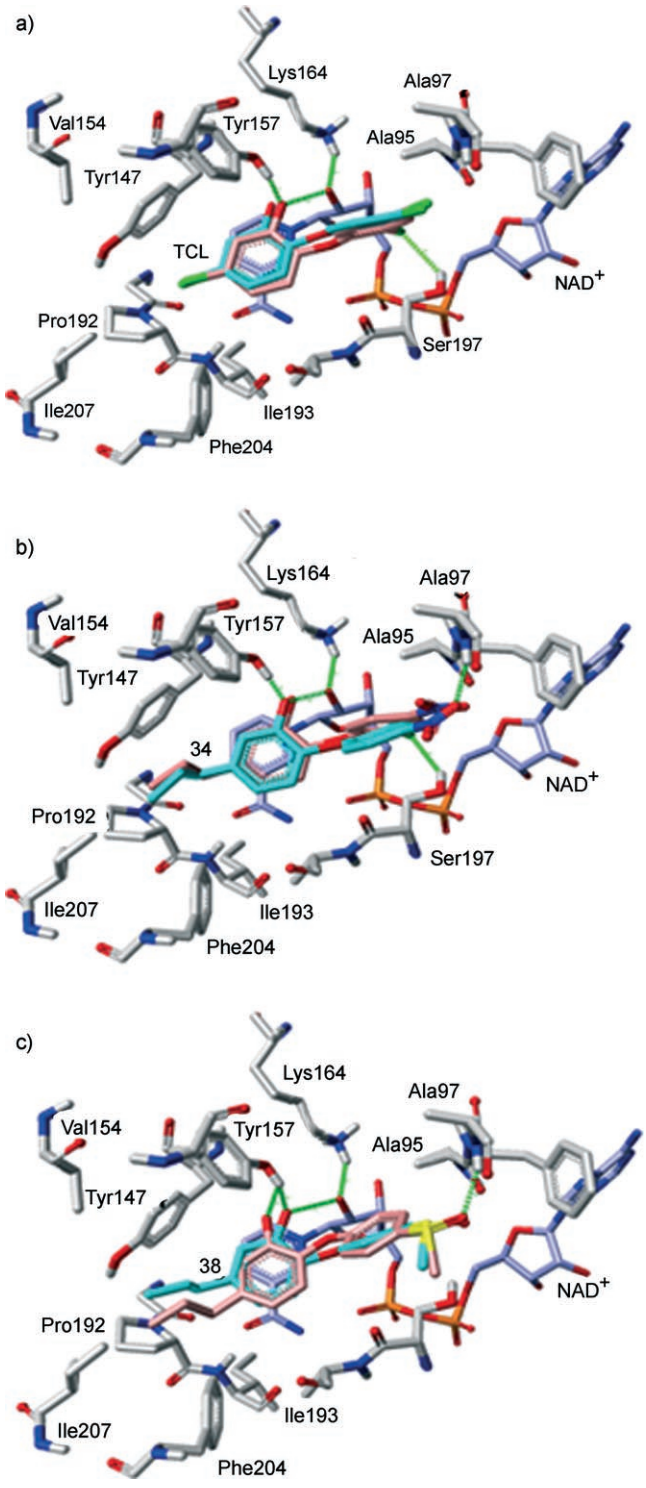


Figure 1. Comparison of ligand-bound crystal structures of *Ba*ENR and the predicted GOLD docking conformation: side chains (gray), NAD^+ (blue-gray, behind the ligand); a) The triclosan crystal structure (coral) and GOLD docking conformation for triclosan (cyan); b) Compound **11** crystal structure (coral) and GOLD conformation (cyan); c) Compound **43** crystal structure (coral) and the GOLD conformation (cyan). Hydrogen bonding (green) is shown between the ligands and Tyr 157, as well as between the 2′-hydroxy of NAD^+ with the 2-hydroxy and the ether linkage of the ligands. Also shown is a hydrogen bond between 2′-chloro of triclosan and Ser 197.

occur within the ring A pocket, further explaining the experimental results.

From the crystal structure shown in Figure 1, it is clear that hydrophobic residues dominate the region surrounding ring A. Sullivan et al. recently reported potent inhibition of ENR (InhA) from *Mycobacterium tuberculosis* by triclosan analogues bearing long aliphatic chains at the R^1 position.[43,51] Because the ring A pocket in *Ba*ENR is much smaller than in InhA, numerous aliphatic chain lengths (2–8 carbons) were evaluated by GOLD docking prior to synthesis to optimize the chain length. We are confident that GOLD docking can accurately predict the binding conformation of these diphenyl ether analogues; an overlay of the predicted GOLD docking conformation and the crystal structure of triclosan show that they are in very good agreement (RMSD = 0.32 Å) (Figure 1 a). The best result from the docking studies (conformation and scoring), where R^1 is *n*-propyl (compound **27**), was synthesized and tested. The IC_{50} value of **27** was higher than **55**, presumably because it could not saturate the *Ba*ENR active site under the experimental concentrations. Sparing solubility made accurate determination of IC_{50} values difficult; however, the *n*-propyl derivative **27** had a MIC value similar to that of compound **55**. We chose to expand two series of compounds: the first was based on a chloro group at R^1 of ring A (**55**), and the second was based on the *n*-propyl group at R^1 (in **27**).

Substitutions on ring B

Preliminary structure–activity relationship (SAR) studies on ring B involved the modification of R^3–R^5, while R^1 (Cl) and R^2 (OH) of ring A remained unchanged. The goal was to increase the binding affinities of these compounds by increasing the hydrogen bonding interactions in the ring B pocket, specifically to the backbone of Ala 95–Ala 97; from analysis of the modeling results and crystal structure presented in Figures 1 and 2, these residues appear to be potential hydrogen bond donors/acceptors. Several functional groups capable of hydrogen bonding were introduced at the 4′-position of ring B (R^5) (Table 2). Introduction of a hydroxy group did not improve the activity (compound **3**). Substitution with an amino group (compound **20**) led to improved antibacterial activity, but weaker inhibition of *Ba*ENR (IC_{50} = 7 μM). Acetylation or sulfonylation of the amino group was not effective in improving the *Ba*ENR activity (compounds **18**, **19**, and **22**).

Compound **6**, where R^3 = Cl, and $R^5 = NO_2$, a known inhibitor of malarial ENR from *P. falciparum*,[41] was the best inhibitor in this series (IC_{50} = 290 nM and MIC = 3.1 μg mL^{-1}). The nitro group decreases the electron density of ring B, but perhaps more importantly, it adds two potential hydrogen bond acceptors. Compound **7**, where R^5 = Ph, is more active against the bacterium (MIC = 1.9 μg mL^{-1}) than triclosan, but not as effective against *Ba*ENR, indicating a nonspecific or alternative mode of action, or that the biphenyl ring improves bacterial membrane penetration. Similarly, the symmetrical triaryl ethers **32** and **33** were ineffective against *Ba*ENR, even though **33** showed improved antibacterial activity.

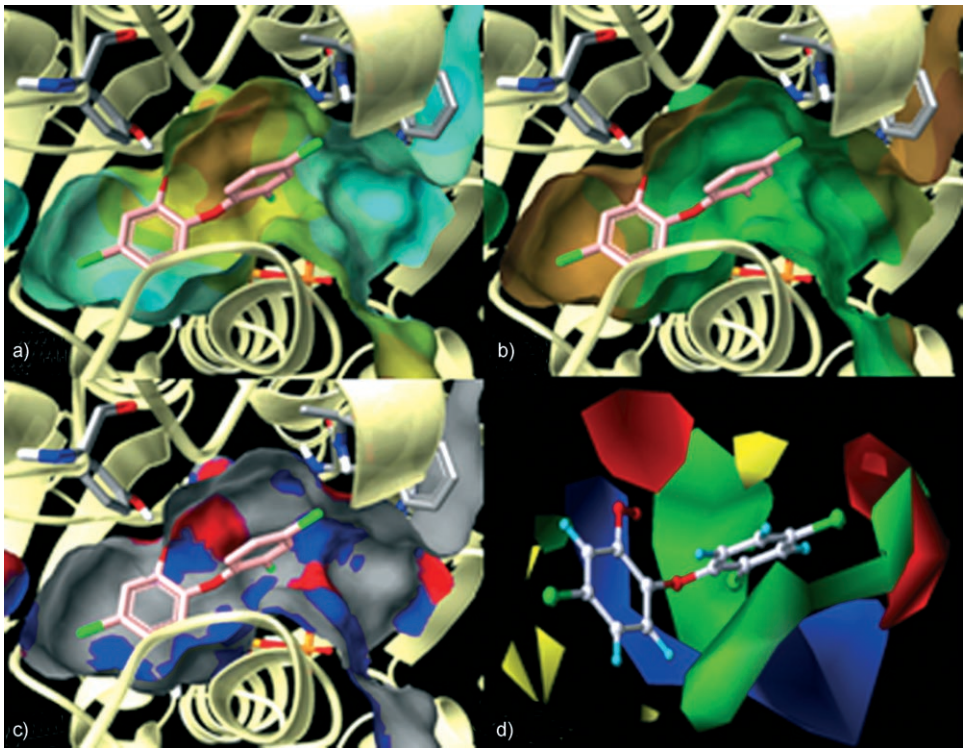


Figure 2. Surface maps of the *Ba*ENR active site: a) Electrostatic potential surface map showing regions of moderate positive charge (orange/yellow) and areas of neutral charge (cyan) on *Ba*ENR; b) Lipophilic map with a brown to green scale, where brown depicts the more hydrophobic areas; c) Hydrogen bonding map where the blue and red areas indicate places for either hydrogen bonding acceptors or donors, respectively; d) Steric and electrostatic fields from the CoMFA based on IC_{50} values. Steric fields are green and yellow, indicating regions of favorable and unfavorable steric expansion. Electrostatic fields are red and blue, indicating preferred regions of negative and positive charge. Surface maps were generated using the Benchware software and CoMFA fields were generated using Sybyl 7.2, (both softwares from Tripos, Inc., St. Louis, MO).

Considerable improvement in the IC_{50} values of compound **6** over **4** (0.3 μM versus 7.2% inhibition at 1 μM) suggests that a 2′-chloro group considerably increases binding affinity. From the crystal structure of triclosan bound to *Ba*ENR (Figure 1a), the hydroxy group of Ser197 is 2.6 Å from the 2′-chloro group, which is sufficiently close for a favorable halogen–oxygen interaction,[52] and also 2.7 Å from one of the oxygen atoms of the bridging phosphate group of NAD^+. The improved activity of the 2′-chloro compounds may be due to this hydrogen bond network, rendering ring B in a "locked" orientation, favorably positioning R^5 substituents to form hydrogen bonds with the backbone of Ala97. Hydrogen bonding to Ser197 (OH) may also stabilize positioning of the substrate binding or "flipping" loop, which is flexible in a number of organisms.[16,17,46]

To improve the solubility of these diphenyl ethers, we synthesized heteroaromatic ring B analogues of **55**, such as the pyridine and pyrazine (data shown in Supporting Information). These compounds failed to show promising activity; acetamidopyridine derivative **24** gave a moderate IC_{50} value (IC_{50} = 4.1 μM), but proved to be inactive against the bacterium (MIC > 111.5 μg mL^{-1}), and so no further such modifications were investigated.

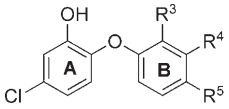
Combining the SAR data of rings A and B, we synthesized compounds in which ring A was the more hydrophobic 2-hydroxy-4-*n*-propylphenyl moiety (**27**, Table 1), and ring B was modified to provide additional hydrogen bond donors/acceptors, both at the R^4 and the R^5 positions, allowing close interactions with the residues in the region of Ala97, and positioning the ring B substituents toward the entrance of the active site, closer to the protein surface.

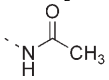
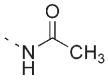
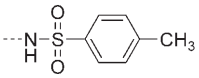
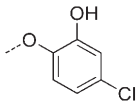
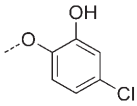
The *n*-propyl series of compounds showed an overall improvement in both the IC_{50} and MIC values over those listed in Tables 1 and 2 (Table 3). Introduction of small, hydrophilic hydrogen bond acceptor groups, which have the ability to withdraw electron density from ring B, increases activity. The best results were obtained when ring B contained a nitro or a cyano substituent (**8**–**12**, Table 3). As discussed above, the presence of a 2′-chloro group contributed to the binding affinity of these inhibitors (**8** versus **11**). Compounds with either an amide (in **15**), or methyl ketone (in **40**) group also had inhibitory activities similar to triclosan. The compounds with an electron-rich amino group at the R^5 position were found to be weak inhibitors of *Ba*ENR (e.g. **8** versus. **21**), consistent with the results of compound **55** versus **20** (Table 2).

The correlation of inhibitory activities with the electron-donating or -withdrawing ability of R^5 substituents, such as NH_2 (compound **21**) and NO_2 (compound **8**), does not hold for the sulfur-containing derivatives. Improved IC_{50} values were seen with the more electron-donating methylthioether group (**41**, IC_{50} = 0.6 μM), while compounds bearing relatively stronger electron-withdrawing groups were only moderately active (methylsulfoxide **43**, IC_{50} = 3.6 μM, or methylsulfone **44**, IC_{50} = 2.2 μM). An electrostatic effect and a steric contribution and/or orientation of the hydrogen bond acceptor in the active site contribute to the inhibitory activity of these compounds. The different geometry of a sulfone compared with a nitro group means that the oxygen atoms are positioned differently, leading to distinct hydrogen bonding capabilities. It is notable here that methyl ketone **40**, which is a close structural analogue of methylsulfoxide **43**, exhibits improved inhibitory activity against *Ba*ENR and ΔANR *B. anthracis* (IC_{50} = 0.8 μM, MIC = 13.5 μg mL^{-1}).

To better understand the binding interactions of these inhibitors at the active site, we determined the X-ray crystal structures of inhibitors **11** and **43** bound to *Ba*ENR in the presence

Table 2. *Ba*ENR inhibition and antibacterial activity for modification of ring B.



Compound	R^3	R^4	R^5	IC_{50} [μM] or % inhibition at 1 μM	MIC[b] [μg mL^{-1}]
Triclosan	Cl	H	Cl	0.6 ± 0.0	3.1
55	H	H	H	0.5 ± 0.1	32
3	H	H	OH	2.6%	>64
20	H	H	NH_2	7.1 ± 1.2	12
18	H	H		1.6%	111
19		H	H	0%	111
22	H	H		>12[a]	4.9
4	H	H	NO_2	7.2%	3.3
5	NO_2	H	H	7.7 ± 0.7	13.3
6	Cl	H	NO_2	0.3 ± 0.0	<0.1–3.1
7	H	H	Ph	>6.25	1.9
32	H	H		15.5%	>145
33	H		H	9.9%	2.3

[a] Saturation with inhibitor was not obtained over the concentration range tested. The percent inhibition of *Ba*ENR showed a linear response to increasing inhibitor concentrations. [b] MIC values are against ΔANR *B. anthracis*.

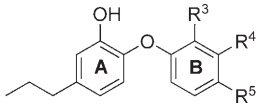
of NAD^+ (2.3 Å resolution, Figure 1b and c), and compared them to the structure of *Ba*ENR in complex with triclosan. As expected from modeling studies, the binding conformations of these inhibitors were nearly identical to that of triclosan. The most significant difference between the structures was the interaction between R^5 and the protein. Improved activity of compound **11** can be attributed to the additional hydrogen bond between the 4′-nitro group and Ala 97(NH) (~2.2 Å), accurately predicted by the GOLD docking results, as well as the proposed orientation of the 2′-chloro group within hydrogen bonding distance to the side chain of Ser 197. Figure 1b is an overlay of the X-ray crystal structure of compound **11** and its GOLD docking conformation in the active site of *Ba*ENR (RMSD = 0.45 Å). Figure 1c is an overlay of the X-ray crystal structure of compound **43** and its GOLD docking conformation in the active site of *Ba*ENR (RMSD = 0.69 Å). Again, there is very good agreement between the two, as well as the correct prediction of the hydrogen bond between the sulfoxide and Ala 97(NH) (~1.8 Å).

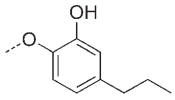
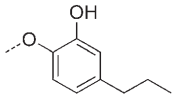
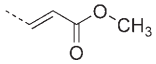
Ring B modifications at the *meta* position (R^4) involved the addition of heteroatoms close to the aromatic ring, including compounds **45** (NMe_2), **46** (CF_3), and **38** (CH_2OH). Only compound **46** showed improved activities against *Ba*ENR and the bacterium. Compounds **36** and **34**, where R^4 = Ph, were more active toward the bacterium than triclosan itself (MIC = 1.9 and 1.2 μg mL^{-1} respectively), but exhibited weaker activities against *Ba*ENR. It is well documented that the ENR from various organisms has a very flexible substrate binding loop;[46,53] the bulky phenyl substituent may induce a shift in this loop, opening up the active site, and leading to a decrease in the binding affinity of the compound for *Ba*ENR.

Compounds **37**–**39** and **48**–**51** are closely related R^4 ester and acid analogues. As previously noted, modeling and structural analysis suggests that hydrogen bonds may be formed between ring B substituents and the residues surrounding Ala 97 in the active site of *Ba*ENR. Hydrogen bonding is optimal when the acceptor/donor R^4 group is 1–2 atoms away from ring B, as seen in compounds **37**–**39**, while longer chain substituents result in weaker inhibition (**48**–**51**). Although this modification proved disappointing, these results indicate that esters are better than acids at inhibiting *Ba*ENR (**37** versus **39**, **49** versus **51**).

While some of the 3′-substituted analogues show IC_{50} values near 1 μM, the majority had little activity against *Ba*ENR (less than 20% inhibition at 1 μM). This is in contrast to the 4′-substituted analogues, which generally had IC_{50} values $\leq$3.6 μM. MIC values suggest that 3′-substituted compounds have greater antibacterial activity than analogous 4′-derivatives (**34** versus **35**, Table 3). Figure 3 summarizes the SAR of the di-

Table 3. *Ba*ENR inhibition and antibacterial activity for modification of ring B, where ring A is a 2-hydroxy-4-propylphenyl group.



Compound	R^3	R^4	R^5	IC_{50} [μM] or % inhibition at 1 μM	MIC[c] [μg mL^{-1}]
Triclosan				0.6 ± 0.0	3.1
27	H	H	H	> 0.8[a]	22.8
8	H	H	NO_2	1.1 ± 0.1	3.4
9	H	NO_2	H	> 0.8[a]	1.7
10	NO_2	H	NO_2	3.6 ± 0.8	4
11	Cl	H	NO_2	0.5 ± 0.0	1.9–3.1
12	Cl	H	CN	> 0.8[a]	1.8
15	Cl	H	$C(=O)NH_2$	1.1 ± 0.1	30.6
40	H	H	C(=O)Me	0.8 ± 0.1	13.5
43	H	H	S(=O)Me	3.6 ± 0.3	116
44	H	H	SO_2Me	2.2 ± 0.3	61.3
42	H	H	CH(OH)Me	17.1 %	54.5
21	H	H	NH_2	8.8 ± 1.0	> 97
41	H	H	SMe	0.6 ± 0.0	13.5
45	H	$N(Me)_2$	H	12.6 %	13.6
46	H	CF_3	H	1.24	7.4
37	H	CO_2Me	H	2.0 ± 0.3	3.6
39	H	CO_2H	H	9.2 %	6.8
38	H	CH_2OH	H	20.3 ± 1.3	12.9
36	H	Ph	H	0.5 ± 0.1[b]	1.9
34	H		H	20.6 %	1.2
35	H	H		10.0 %	> 151
48	H		H	> 6.25	2
49	H	$CH_2CH_2CO_2Me$	H	7.5 ± 1.9	7.9
50	H	$CH=CHCO_2H$	H	17.9 %	14.9
51	H	$CH_2CH_2CO_2H$	H	17.6 %	60.1
13	H	CH_2CH_2Br	H	6.0 ± 1.5	1.7

[a] Saturation with inhibitor was not obtained over the concentration range tested. The percent inhibition of *Ba*ENR showed a linear response to increasing inhibitor concentrations. [b] 100% inhibition was not observed. The response of the enzyme to inhibitor showed maximum saturation at ~50% inhibition. [c] MIC values are against ΔANR *B. anthracis*.

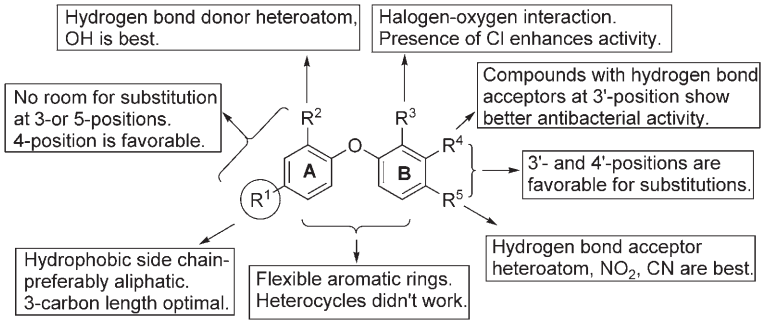


Figure 3. Summary of the SAR of diaryl ether inhibitors of *Ba*ENR.

phenyl ethers studied, showing the key pharmacophore functionalities required for effective *Ba*ENR inhibition.

Considering the enzyme assay alone, the *n*-propyl R^1 series of compounds show better inhibitory activity against *Ba*ENR than the chloro series shown in Table 2. More importantly, this series has resulted in nine compounds (**8**, **9**, **11**, **12**, **15**, **27**, **36**, **40**, and **41**) that are equipotent or near-equipotent with triclosan. Of these, **15**, **40**, and **41** have the potential for further structural modification and expansion. Compounds where $R^3 = Cl$ have also shown increased activity against *Ba*ENR. Interestingly, we observed a similar improvement in the binding affinity of 2-pyridone derivatives with *Ba*ENR in a recent study;[54] hence, introduction of a chloro group to **40** and **41** is predicted to improve their inhibitory activities. Additionally, carboxamide **15**, methyl ketone **40**, and thioether **41** possess R^5 functionalities that are amenable to further derivatization and therefore useful in the design of the next generation of compounds.

CoMFA maps and future design implications

GOLD accurately predicted the binding conformations of simple substitutions; however, it is unable to account for the flexibility in the *Ba*ENR substrate binding loop. A ligand-based approach, 3D-QSAR, or comparative molecular field analysis (CoMFA), is needed for further drug design. We performed a CoMFA on the results obtained against *Ba*ENR, presented in Tables 1–3 and the Supporting Information. The CoMFA model has $q^2 = 0.831$, and $r^2 = 0.929$. Figure 2d shows the steric and electrostatic fields generated from the CoMFA. The R^2 hydroxy group and R^5 substituent protrude favorably into negatively charged regions (red). The CoMFA map suggests that steric expansion of R^4 and R^5 would be beneficial, while ring A expansion should be limited, as shown by the yellow regions here. The large green region behind the molecule could potentially be misleading; from the crystal structure, we know this to be the NAD^+ binding region. Modifications that occupy this region are not likely to be beneficial because triclosan and its analogues co-bind with NAD^+ for optimal binding energy.[37,46,55] The CoMFA model provides insight for the further structural expansion of these aryl ethers and will be used to predict the activities of the next generation of compounds.

Antibacterial testing of lead compounds

Table 4 shows the MIC and MBC values of **6** and **11** against several bacterial pathogens compared to ciprofloxacin. The broth microdilution method (National Committee for Clinical Laboratory Standards) was validated using a panel of ATCC strains of bacterial pathogens with known MIC values for ciprofloxacin, and then employed to determine the MIC and MBC values for **6** and **11**. The results show that **6** and **11** are equally active against Sterne and ΔANR strains of *B. anthracis*. These compounds also have noticeable activity against both Gram(+) and Gram(−) bacteria, suggesting their potential expansion to a more broad-spectrum application. A notable exception is their lack of activity against *P. aeruginosa,* which was reported for triclosan also,[56] and may be explained by bacterial efflux pump activity.[29] Both compounds exhibited impressive activity against MRSA (MIC = 0.3 μg mL^{-1}), and an MBC/MIC ratio of 1.6–1.9, compared with a ratio of ~20 for methicillin-sensitive *S. aureus* (MSSA); the comparable activities against MRSA and MSSA are consistent with previous reports.[56] Should the 10-fold increase in antibacterial activity against MRSA over MSSA be reproducible in further tests against multiple strains of each, it might be interesting to pursue further mechanistic investigations.

Previous studies have indicated that the bactericidal activity of triclosan against *S. aureus* (both MRSA and MSSA) may be unrelated to specific enzyme inhibition but rather involve multiple bacterial targets.[57] It is likely that compounds **6** and **11** target FabI in *S. aureus*, however, in the absence of an X-ray crystal structure of ENR from *S. aureus*, this increased activity must be explained by secondary structures. Sequence alignment of ENR from *S. aureus* and *B. anthracis* indicates 62% homology, with all residues in the substrate binding loop (residues 190–210), and all residues within 5 Å radius of the active site fully conserved between species with the exception of a single residue, where Met 99 (*S. aureus* ENR) is substituted by Arg 99 (*Ba*ENR). Arg 99 is located near the surface of *Ba*ENR, with the arginine side chain partially covering the entrance to the active site. Substitution with methionine (*S. aureus* ENR) is likely to leave the active site more accessible for the ligands, and could partially explain the increased activity of the biaryl ethers against *S. aureus* compared with *B. anthracis*. Further investigation is needed to assess the strain specificity and ENR pathway dependence of these MBC/MIC results. Preliminary cytotoxicity evaluation of **6** and **11** showed an EC_{50}/MIC ratio of approximately 15–20 against HeLa cells (footnote, Table 4). Further testing is needed to validate these values and assess whether structural modifications are necessary to minimize toxicity.

Conclusions

A number of novel aryl ethers, including triphenyl ethers and heteroaromatic analogues, have been prepared and tested for

Table 4. MIC and MBC values of ENR inhibitors against *B. anthracis* and other bacterial pathogens.

Bacteria	Cipro[a] MIC[b]	Compound **6**[e] MIC	MBC	MBC/MIC	Compound **11**[e] MIC	MBC	MBC/MIC
ΔANR[c]	0.1 ± 0.0	2.2 ± 0.4	3.1 ± 1.1	1.4	2.2 ± 0.0	3.9 ± 0.8	1.8
Sterne[d]	0.1 ± 0.0	1.9 ± 0.3	3.9 ± 0.8	2.1	1.9 ± 0.0	3.9 ± 0.8	2.1
S. aureus	0.4 ± 0.0	0.1 ± 0.1	2.9 ± 1.2	20.9	0.1 ± 0.1	2.9 ± 1.2	20.9
MRSA	0.5 ± 0.2	0.3 ± 0.3	0.4 ± 0.1	1.6	0.3 ± 0.3	0.5 ± 0.2	1.9
E. fecalis	0.7 ± 0.1	4.4 ± 0.8	31.3 ± 6.3	7.1	4.4 ± 0.5	12.5 ± 0.0	2.9
VRE	0.6 ± 0.2	5.7 ± 0.6	37.5 ± 12.5	6.6	5.7 ± 0.3	15.6 ± 3.1	2.7
L. monocytogenes	0.9 ± 0.3	2.8 ± 0.3	14.1 ± 3.9	5.0	2.8 ± 0.9	14.1 ± 3.9	5.0
P. aeruginosa	0.3 ± 0.1	> 25	NT		> 25	NT	
K. pneumoniae	0.5 ± 0.1	1.8 ± 0.4	12.5	7.1	1.8 ± 0.9	8.3 ± 2.1	4.7
E. coli	0.1 ± 0.0	0.3 ± 0.1	12.50	48.0	0.3 ± 0.2	1.2 ± 0.4	4.5

[a] Drug concentrations = μg mL^{-1}. Results = mean ± SEM, $n = 3$. [b] MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; Bactericidal drug: MBC/MIC ≤ 4. [c] Plasmid-negative strain of *B. anthracis*, lacking both pXO1 and pXO2 plasmids. [d] Sterne strain of *B. anthracis* that contains pXO1 (toxin production) but lacks pXO2 (capsule). [e] The EC_{50} against the HeLa cell line is 29.3 μg mL^{-1} for **6** and 41.4 μg mL^{-1} for **11**. EC_{50} against the MHS cell line is 9.9 μg mL^{-1} for **6** and 22.6 μg mL^{-1} for **11**.

inhibition of purified *Ba*ENR and cultured ΔANR *B. anthracis*. These efforts have led to an improved understanding of the enzyme active site, and provided clear SAR data for these inhibitors. The X-ray crystal structures coupled with molecular modeling studies have demonstrated the importance of hydrophobic interactions of substituents located at the R^1 position with the enzyme active site, and underscore the H-bonding contribution of the *ortho* (R^3) and *para* (R^5) substituents on ring B with Ser 197 (OH) and Ala 97. In particular, the importance of the *ortho*-chloro group on ring B for optimized activity warrants further investigation. These results highlight the biological activity's sensitivity towards changes in electron distribution in either ring of the 2-phenoxyphenol. For example, although replacement of a chloro for a *n*-propyl group at R^1 improved activity, simultaneous and complementary substitution is needed on ring B. Structure-based design efforts are ongoing with a focus on R^5 modifications and linking groups such as thioethers, amides, and esters, as well as nitro group modification.

Several compounds have been synthesized that exhibit structural diversity and improved antibacterial activity over triclosan, and offer an opportunity to identify new inhibitory pathways and drug candidates. Encouraging inhibitory activities of these compounds against a number of pathogens suggests potential for broad-spectrum applications. The intriguing activity shown by compounds **6** and **11** against MRSA provides potential leads against a serious and increasingly common pathogen. To advance these compounds further, pharmacokinetic and drug metabolism studies are planned.

Experimental Section

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400, and an AVANCE-400 spectrometer with TMS as an internal standard. HRMS data were performed using a Q-TOF-2TM (Micromass). Preparative TLC was performed with 1000 μm silica gel GF plates (Analtech). Column chromatography was performed using 40–60 mesh silica gel (Merck). HPLC was carried out on an ACE AQ columns (100×4.6 and 250×10 mm), with detection at 254 nm on a Shimadzu SPD-10 A VP detector; flow rate = 2.0–3.5 mL min^{-1}; from 10% CH_3CN in water to 100% CH_3CN with 0.05% TFA. Compound **54** was commercially available from Sigma–Aldrich. Compounds **53**, **55**,[37] and **28**[26,37] were synthesized according to published methods.

Synthesis of inhibitors

General Methods: **Method A**: A solution of aryl halide (1 mmol), phenol (1 mmol), and K_2CO_3 (2–4 mmol) in DMSO (1.5 mL) were heated to 100 °C under nitrogen until completion (8–12 h). The reaction was cooled to RT, diluted with EtOAc, and washed with aq NaOH (5%). The aqueous layer was further extracted with EtOAc, and the combined organic layers washed with brine. The organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo to give the crude product, which was purified by chromatography. **Method B**: KO*t*Bu (1.1 mmol) was added to a solution of phenol (1 mmol) in DMF (1.75 mL) in one portion, and the reaction heated at 45 °C under mild vacuum (2 h). The reaction was cooled to RT, and treated with aryl halide (1 mmol) and $(CuOTf)_2$·PhH (0.05 mmol), then heated to reflux (16–20 h). The reaction was cooled to RT, diluted with EtOAc, and filtered over Celite. The filtrate was washed with aq NaOH (5%), extracted with EtOAc, and the combined organic layers washed with brine. The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo to give the crude product, which was purified by chromatography. **Method C**: A suspension of arylboronic acid (1–2 mmol), phenol (1 mmol), $Cu(OAc)_2$ (2–5 mmol), Et_3N (5–10 mmol) and powdered molecular sieves (5 Å) in CH_2Cl_2 (10 mL) was stirred open to the air until completion (3–16 h). The reaction mixture was diluted with EtOAc, filtered over Celite, and the filtrate was washed with aq NaOH (5%). The aqueous layer was extracted with EtOAc, and the combined organic layers were washed with brine. The organic phase was dried (Na_2SO_4), filtered and concentrated in vacuo to give the crude product, which was purified by chromatography. **Method D**: A solution of BBr_3 (2–8 mmol, 1.0 M in CH_2Cl_2) was added to a solution of diphenyl ether (1 mmol) in anhyd CH_2Cl_2 (4 mL) under nitrogen at −78 °C and stirred (1 h) before warming to RT and stirring until completion (3–8 h). The reaction was cooled to −78 °C and quenched with MeOH. The reaction was concentrated in vacuo then redissolved in EtOAc, washed with 10% aq $NaHCO_3$, water and brine; the aqueous layer was extracted with EtOAc (×2). The combined organic layers were then dried (Na_2SO_4), filtered, concentrated in vacuo and purified by chromatography.

4-Phenoxybenzene-1,3-diol (1): Method B was used to prepare the intermediate **1a** from 2,4-dimethoxyphenol (1.00 g, 6.5 mmol), KO*t*Bu (0.87 g, 7.8 mmol), $(CuOTf)_2$·PhH (0.17 g, 0.3 mmol) and iodobenzene (1.59 g, 7.8 mmol) in 50% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (10% EtOAc/hexanes) gave **1** as a viscous brown oil (80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.86 (s, 1H), 5.56 (s, 1H), 6.36 (dd, J = 9.0, 3.0 Hz, 1H), 6.58 (d, J = 2.8 Hz, 1H), 6.83 (d, J = 8.7 Hz, 1H), 7.08 (d, J = 7.6 Hz, 2H), 7.09 (t, J = 7.4 Hz, 1H), 7.33 ppm (t, J = 7.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 103.4, 107.3, 116.4, 120.6, 122.7, 129.5, 136.3, 148.0, 152.5, 157.3 ppm; HRMS (ESI+): *m/z* calcd for $C_{12}H_{10}O_3$ ($[M+H]^+$): 201.0557, found: 201.0556.

5-Nitro-2-phenoxyphenol (2): Method A was used to prepare the intermediate **2a** from phenol (0.66 g, 7.0 mmol), 1-fluoro-2-methoxy-4-nitrobenzene (1.12 g, 7.0 mmol) and K_2CO_3 (1.80 g, 12.8 mmol) in 48% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (15% EtOAc/hexanes) gave **2** as a greenish yellow oil (75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.53 (br s, 1H), 7.08–7.14 (m, 3H), 7.26 (t, J = 7.6 Hz, 1H), 7.43 (t, J = 7.6 Hz, 2H), 7.70 (d, J = 2.8 Hz, 1H), 7.97 ppm (dd, J = 4.0, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 112.5, 115.3, 118.8, 120.1, 124.9, 130.0, 140.7, 143.9, 154.5, 152.4 ppm; HRMS (ESI−): *m/z* calcd for $C_{12}H_9NO_4$ ($[M-H]^-$): 230.0459, found: 230.0458.

5-Chloro-2-(4-hydroxyphenoxy)phenol (3): Method B was used to prepare the intermediate **3a** from 4-chloro-2-methoxy-phenol (1.00 g, 6.3 mmol), KO*t*Bu (0.85 g, 7.6 mmol), $(CuOTf)_2$·PhH (0.17 g, 0.3 mmol) and 4-iodoanisole (1.80 g, 7.6 mmol) in 49% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (10% EtOAc/hexanes) gave **3** as a colorless oil (82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.68 (s, 1H), 5.72 (s, 1H), 6.70 (d, J = 8.8 Hz, 1H), 6.79 (dd, J = 8.0, J = 2.0 Hz, 1H), 6.84 (dd, J = 7.0, J = 2.0 Hz, 2H), 6.94 (dd, J = 7.0, J = 2.0 Hz, 2H), 7.04 ppm (d, J = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 115.8, 116.1, 117.4, 119.8, 119.9, 128.2, 143.3, 147.1, 149.1, 151.7 ppm; HRMS (ESI−): *m/z* calcd for $C_{12}H_9ClO_3$ ($[M-H]^-$): 235.0168, found: 235.0166.

5-Chloro-2-(4-nitrophenoxy)phenol (4): Method A was used to prepare the intermediate **4a** in 96% yield, as described in the synthesis of **20**, and method D was used to convert it to the title compound. Purification by flash chromatography (3% MeOH/$CHCl_3$) gave the **4** as a brown solid (70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.42 (s, 1H), 6.95 (s, 2H), 7.14–7.02 (m, 3H), 8.26 ppm (d, J = 9.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 116.5, 117.3, 120.9, 121.1, 125.7, 131.2, 139.7, 142.9, 147.9, 161.7 ppm; HRMS (ESI−): *m/z* calcd for $C_{12}H_8ClNO_4$ ([M−H]$^-$): 264.0069, found: 264.0069.

5-Chloro-2-(2-nitrophenoxy)phenol (5): Method A was used to prepare the intermediate **5a** in 96% yield, as described in the synthesis of **19**, and method D was used to convert it to the title compound. Purification by flash chromatography (1% MeOH/$CHCl_3$) gave **5** as an off-white solid (76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.89 (dd, J = 4.0, J = 2.0 Hz, 1H), 6.98 (d, J = 4.0 Hz, 1H), 7.12–7.08 (m, 2H), 7.28–7.23 (m, 1H), 7.57–7.53 (m, 1H), 7.94 ppm (dd, J = 4.0, J = 1.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 117.8, 118.9, 120.6, 121.3, 123.9, 125.9, 131.5, 134.6, 140.9, 148.7, 150.0 ppm; HRMS (ESI−): *m/z* calcd for $C_{12}H_8ClNO_4$ ([M−H]$^-$): 264.0069, found: 264.0068.

5-Chloro-2-(2-chloro-4-nitrophenoxy)phenol (6): Method A was used to prepare the intermediate **6a** from 4-chloro-2-methoxyphenol (1.00 g, 6.3 mmol), 2-chloro-1-fluoro-4-nitrobenzene (1.10 g, 6.3 mmol) and K_2CO_3 (1.80 g, 12.6 mmol) in 91% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (3% MeOH/$CHCl_3$) gave **6** as a light yellow solid (79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.73 (s, 1H), 6.97–6.91(m, 3H), 7.14 (d, J = 1.2 Hz, 1H), 8.11 (dd, J = 4.0, J = 1.0 Hz, 1H), 8.41 ppm (d, J = 2.0 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ = 116.1, 117.5, 120.8, 121.0, 123.4, 124.2, 126.3, 131.5, 139.7, 142.9, 147.7, 157.4 ppm; HRMS (ESI−): *m/z* calcd for $C_{12}H_7Cl_2NO_4$ ([M−H]$^-$): 297.9679, found: 297.9679.

2-(Biphenyl-4-yloxy)-5-chlorophenol (7): Method B was used to prepare the intermediate **7a** from 4-chloro-2-methoxyphenol (1.00 g, 6.3 mmol), KO*t*Bu (0.78 g, 6.9 mmol), $(CuOTf)_2$·PhH (0.16 g, 0.3 mmol) and 4-bromobiphenyl (1.47 g, 6.3 mmol) in 69% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (2% EtOAc/hexanes) gave **7** as a white solid (65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.83 (br s, 1H), 6.90 (s, 2H), 7.12 (d, J = 8.8 Hz, 3H), 7.40 (t, J = 7.2 Hz, 1H), 7.49 (t, J = 7.2 Hz, 2H), 7.61 ppm (d, J = 8.0 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 116.3, 117.9, 119.1, 120.3, 126.6, 126.9, 128.3, 128.5, 129.39, 129.2, 136.8, 139.8, 142.9, 147.7, 155.5 ppm; HRMS (ESI+): *m/z* calcd for $C_{18}H_{13}ClO_2$ ([M+H]$^+$): 297.0621, found: 297.0652.

2-(4-Nitrophenoxy)-5-propylphenol (8): Method A was used to prepare the intermediate **8a** from 2-methoxy-4-propylphenol (1.00 g, 6.3 mmol), 1-fluoro-4-nitrobenzene (0.89 g, 6.3 mmol) and K_2CO_3 (1.80 g, 12.6 mmol) in 93% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (10% EtOAc/hexanes) gave **8** as a yellow solid (96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.99 (t, J = 3.2 Hz, 3H), 1.65 (q, J = 3.6 Hz, 2H), 2.59 (t, J = 3.6 Hz, 2H), 6.77 (d, J = 8.0 Hz, 1H), 6.90–6.91 (m, 2H), 7.07 (d, J = 9.2 Hz, 2H), 8.21 ppm (d, J = 1.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.4, 23.9, 37.2, 116.1, 116.8, 120.3, 120.9, 125.6, 138.6, 141.5, 146.9, 162.5 ppm; HRMS (ESI−): *m/z* calcd for $C_{15}H_{15}NO_4$ ([M−H]$^-$): 272.0928, found: 272.0925.

2-(3-Nitrophenoxy)-5-propylphenol (9): Method A was used to prepare the intermediate **9a** from 2-methoxy-4-propylphenol (1.00 g, 6.3 mmol), 1-fluoro-3-nitrobenzene (0.89 g, 6.3 mmol) and K_2CO_3 (1.8 g, 12.6 mmol) in 65% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (15% EtOAc/hexanes) gave **9** as a yellow solid (83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.99 (t, J = 7.2 Hz, 3H), 1.67 (quin, J = 7.6 Hz, 2H), 2.59 (t, J = 7.6 Hz, 2H), 5.37 (s, 1H), 6.76 (dd, J = 4.0, J = 2.0 Hz, 1H), 6.89 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 1.6 Hz, 1H), 7.36 (dd, J = 5.0, J = 2.0 Hz, 1H), 7.50 (t, J = 8.2 Hz, 1H), 7.83 (t, J = 2.0 Hz, 1H), 7.96 ppm (dd, J = 4.0, J = 1.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.4, 23.9, 37.1, 111.5, 116.7, 117.3, 119.6, 120.8, 122.7, 129.9, 139.2, 141.0, 146.9, 148.8, 157.9 ppm; HRMS (ESI−): *m/z* calcd for $C_{15}H_{15}NO_4$ ([M−H]$^-$): 272.0928, found: 272.0926.

2-(2,4-Dinitrophenoxy)-5-propylphenol (10): Method A was used to prepare the intermediate **10a** from 2-methoxy-4-propylphenol (0.50 g, 3.0 mmol), 1-fluoro-2,4-dinitrobenzene (0.56 g, 3.0 mmol) and K_2CO_3 (0.84 g, 6.0 mmol) in 93% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (10% EtOAc/hexanes) gave **10** as a yellow solid (81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.95 (t, J = 7.2 Hz, 3H), 1.65 (quin, J = 7.2 Hz, 2H), 2.59 (t, J = 7.2 Hz, 2H), 5.72 (s, 1H), 6.83 (d, J = 8.2 Hz, 1H), 7.02 (s, 1H), 7.06–7.04 (m, 1H), 7.18–7.13 (m, 1H), 8.36 (dd, J = 4.0, J = 3.0 Hz, 1H), 8.84 ppm (t, J = 2.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 13.3, 24.0, 36.9, 117.4, 117.5, 120.8, 121.2, 121.6, 127.6, 128.7, 136.2, 137.8, 141.2, 142.9, 144.8, 146.8, 155.4 ppm; HRMS (ESI−): *m/z* calcd for $C_{15}H_{14}N_2O_6$ ([M−H]$^-$): 317.0779, found: 317.0776.

2-(2-Chloro-4-nitrophenoxy)-5-propylphenol (11): Method A was used to prepare the intermediate **11a** from 2-methoxy-4-propylphenol (0.50 g, 3.0 mmol), 2-chloro-1-fluoro-4-nitrobenzene (0.53 g, 3.0 mmol) and K_2CO_3 (0.84 g, 6.1 mmol) in 93% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (10% EtOAc/hexanes) gave **11** as a pale yellow solid (82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.97 (t, J = 7.2 Hz, 3H), 1.66 (quin, J = 7.6 Hz, 2H), 2.57 (t, J = 7.6 Hz, 2H), 5.55 (s, 1H), 6.77 (dd, J = 4.0, J = 2.0 Hz, 1H), 6.91–6.89 (m, 3H), 8.02 (dd, J = 5.0, J = 2.0 Hz, 1H), 8.33 ppm (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.4, 23.9, 37.1, 115.3, 117.1, 120.2, 121.1, 123.3, 123.5, 125.9, 138.5, 141.9, 142.1, 146.7, 158.3 ppm; HRMS (ESI−): *m/z* calcd for $C_{15}H_{14}ClNO_4$ ([M−H]$^-$): 306.0539, found: 306.0541.

3-Chloro-4-(2-hydroxy-4-propylphenoxy)benzonitrile (12): Method A was used to prepare the intermediate **12a** from 2-methoxy-4-propylphenol (1.00 g, 6.3 mmol), 3-chloro-4-fluorobenzonitrile (0.94 g, 6.0 mmol) and K_2CO_3 (1.7 g, 12.0 mmol) in 80% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (10% EtOAc/hexanes) gave **12** as a white crystaline solid (79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, J = 2.0 Hz, 1H), 7.47 (dd, J = 4.0, J = 2.0 Hz, 1H), 6.93–6.87 (m, 3H), 6.78–6.75 (m, 1H), 5.42 (s, 1H), 2.58 (t, J = 7.6 Hz, 2H), 1.67 (quin, J = 7.6 Hz, 2H), 0.98 ppm (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.4, 23.9, 37.1, 106.3, 116.4, 117.0, 120.1, 120.9, 124.1, 131.8, 133.8, 138.5, 141.7, 146.9, 156.9 ppm; HRMS (ESI−): *m/z* calcd for $C_{16}H_{14}ClNO_2$ ([M−H]$^-$): 286.0640, found: 286.0637.

2-[3-(2-Hydroxy-ethyl)phenoxy]-5-propylphenol (13): Method B was used to prepare the intermediate **13a** from 2-methoxy-4-propyl-phenol (1 g, 6.0 mmol) and 3-bromostyrene(1.32 g, 7.2 mmol) (62%). Compound **13a** was treated with BH_3·THF (1 M in anhyd THF, 0.5 equiv) and the reaction was stirred at RT (3 h). The reaction was cooled to 0 °C and treated dropwise with aq NaOH (7 mL, 3 M), then hydrogen peroxide (4 mL, 30%), and stirred at RT (12 h) before work-up. Purification using flash column chromatography (2% MeOH/$CHCl_3$) gave **13b** (68%), and method D was used to convert it to the title compound. Purification by flash chromatography (5% EtOAc/hexanes) gave **13** as a colorless oil (75%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.98 (t, J = 7.2 Hz, 3H), 1.67 (t, J = 7.6 Hz, 2H), 2.57 (t, J = 7.6 Hz, 2H), 3.16 (t, J = 7.6 Hz, 2H), 3.57 (t, J = 7.2 Hz, 2H), 5.47 (s, 1H), 6.69 (dd, J = 4.0 Hz, J = 2.0 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 6.93–6.89 (m, 3H), 6.97 (d, J = 7.2 Hz, 1H), 7.30 ppm (t, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.9, 24.5, 32.6, 37.6, 39.1, 116.0, 116.3, 117.8, 119.1, 120.7, 123.6, 129.9, 140.0, 140.9, 141.0, 147.3, 157.5 ppm; HRMS (ESI−): *m/z* calcd for $C_{17}H_{19}BrO_2$ ($[M-H]^-$): 333.0496, found: 333.0492.

5-Chloro-2-phenoxybenzoic acid (14): Method A was used to prepare the intermediate **14a** from 5-chloro-2-fluorobenzonitrile (1.00 g, 6.4 mmol), phenol (0.66 g, 7.0 mmol) and K_2CO_3 (1.8 g, 12.8 mmol) (75%). A solution of **14a** (0.20 g, 0.9 mmol) in aq NaOH (0.4 mL, 25%) and EtOH (2 mL) was refluxed (20 h), then cooled to RT and acidified with dil HCl; the crude product was isolated by filtration. Purification by flash chromatography (6% MeOH/$CHCl_3$) gave **14** as a white solid (143 mg, 64%). ^{1}H NMR (400 MHz, CD_3OD): δ = 6.95 (m, 3H), 7.1 (t, J = 8.0 Hz, 1H), 7.33 (t, J = 8.0 Hz, 2H), 7.46 (dd, J = 9.0, J = 3.0 Hz, 1H), 7.84 ppm (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 119.6, 123.1, 124.8, 129.6, 131.1, 132.4, 134.4, 156.5, 158.6 ppm; MS (ESI) *m/z*: 249 $[M+H]^+$; HRMS (ESI+): *m/z* calcd for $C_{13}H_9ClO_3$ ($[M+Na]^+$): 271.0133, found: 271.0129.

3-Chloro-4-(2-hydroxy-4-propylphenoxy)benzamide (15): A solution of **12** (0.38 g, 1.3 mmol), hydrogen peroxide (1 mL, 30%) and aq NaOH (0.18 mL, 3 M) in EtOH was stirred at 35 °C (20 h), then acidified with H_2SO_4 (1 N). The organic solvent was removed in vacuo and the resulting suspension diluted with EtOAc. The two phases were separated and the organic phase washed with water, then brine, dried (Na_2SO_4), filtered and concentrated in vacuo to give the crude product. Purification by flash chromatography (3% MeOH/$CHCl_3$) gave **15** as a white solid (65%). ^{1}H NMR (400 MHz, CD_3OD): δ = 0.91 (t, J = 7.6 Hz, 3H), 1.58 (quin, J = 7.6 Hz, 2H), 2.50 (t, J = 7.6 Hz, 2H), 6.62 (d, J = 8.0 Hz, 1H), 6.68 (dd, J = 4.0, J = 2.0 Hz, 1H), 6.82 (d, J = 2.0 Hz, 1H), 6.95 (d, J = 8.0 Hz, 1H), 7.39 (br s, 1H), 7.73 (dd, J = 4.0, J = 2.0 Hz, 1H), 7.97 (br s, 1H), 8.03 ppm (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 115.5, 117.7, 120.2, 121.5, 122.3, 128.3, 129.1, 129.9, 139.7, 141.1, 149.1, 156.3, 166.6 ppm; HRMS (ESI−): *m/z* calcd for $C_{16}H_{16}ClNO_3$ ($[M-H]^-$): 304.0746, found: 304.0743.

5-Chloro-2-phenoxyphenylmethanol (16): A solution of $BF_3{\cdot}Et_2O$ (17 μL, 0.13 mmol) in THF (2 mL) was added slowly to a solution of $NaBH_4$ (0.012 g, 0.31 mmol) and **14** (0.051 g, 0.21 mmol) in THF (1 mL) at RT under an inert atmosphere. The mixture was held at reflux (1 h) before it was cooled to 0 °C, and quenched with water and stirred (10 min). The organic solvent was removed in vacuo, and CH_2Cl_2 was added to the aqueous phase. After stirring (1 h), the organic layer was separated, washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by flash chromatography (18% EtOAc/hexanes) gave **16** as a white solid (39 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.11 (br s, 1H), 4.72 (s, 2H), 6.79 (d, J = 9.0 Hz, 1H), 6.96 (d, J = 8.0 Hz, 2H), 7.12 (t, J = 8.0 Hz, 1H), 7.20 (dd, J = 8.0, J = 2.0 Hz, 1H), 7.34 (t, J = 8.0 Hz, 2H), 7.47 ppm (d, J = 3.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 60.7, 118.6, 119.9, 123.4, 129.0, 129.1, 130.2, 133.9, 153.3, 157.0 ppm; HRMS (ESI+): *m/z* calcd for $C_{13}H_{11}ClO_2$ ($[M]^+$): 234.0448, found: 234.0458.

5-Chloro-2-phenoxybenzamide (17): A solution of **14a** (0.20 g, 0.9 mmol), hydrogen peroxide (0.37 mL, 35%) and aq NaOH (0.36 mL, 3 M) in EtOH (5 mL) was stirred at 30 °C (18 h), then acidified with H_2SO_4 (1 N); the crude product was isolated by filtration. Purification by flash chromatography (60% EtOAc/hexanes) gave **17** as a white solid (171 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.55 (br s, 1H), 6.76 (d, J = 8.0 Hz, 1H), 7.07 (d, J = 6.0 Hz, 2H), 7.26 (t, J = 8.0 Hz, 1H), 7.34 (dd, J = 9.0, J = 3.0 Hz, 1H), 7.42 (t, J = 8.0 Hz, 2H), 7.55 (br s, 1H), 8.24 ppm (d, J = 2.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 119.6, 120.0, 125.4, 129.2, 130.6, 132.4, 133.2, 154.9, 133.2, 165.0 ppm; MS (ESI+): *m/z* 248 $[M+H]^+$; HRMS (ESI+): *m/z* calcd for $C_{13}H_{10}ClNO_2$ ($[M+Na]^+$): 270.0293, found 270.0286.

***N*-[4-(4-Chloro-2-hydroxyphenoxy)phenyl]acetamide (18)**: The intermediate **20a** (0.14 g, 0.6 mmol), prepared as described in the synthesis of **20**, was treated with Ac_2O (0.07 g, 0.7 mmol), catalytic DMAP and Et_3N (0.07 g, 0.7 mmol) in CH_2Cl_2 (2 mL) at 0 °C (3 h), and method D was used to convert it to the title compound. Purification by flash chromatography (3% MeOH/$CHCl_3$) gave **18** as a white solid (92%). ^{1}H NMR (400 MHz, CD_3OD): δ = 2.11 (s, 3H), 7.00–6.78 (m, 5H), 7.52–7.45 ppm (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ = 21.9, 116.4, 116.9, 119.0, 120.6, 121.2, 121.3, 124.2, 128.6, 133.1, 142.6, 149.2, 153.6, 169.8 ppm; HRMS (ESI−): *m/z* calcd for $C_{14}H_{12}ClNO_3$ ($[M-H]^-$): 276.0433, found: 276.0432.

***N*-[2-(4-Chloro-2-hydroxyphenoxy)phenyl]acetamide (19)**: Method A was used to prepare the intermediate **5a** from 4-chloro-2-methoxyphenol (1.00 g, 6.3 mmol), 1-fluoro-2-nitrobenzene (0.89 g, 6.3 mmol) and K_2CO_3 (1.80 g, 12.6 mmol) in 96% yield. A mixture of **5a** (1.70 g, 6.1 mmol) and 10% Pd/C (0.30 mg) in EtOH (24 mL) was stirred at RT under H_2 (6 h). The reaction was filtered through Celite, the filter pad was washed with EtOH and the filtrate was concentrated in vacuo. Purification by flash chromatography (10% EtOAc/hexane) gave **19a** as a brown liquid (0.82 g, 92%). A solution of **19a** (0.12 g, 0.47 mmol) in CH_2Cl_2 (3 mL) was treated with Ac_2O (0.06 g, 0.56 mmol), catalytic DMAP (0.006 g) and Et_3N (0.06 g, 0.56 mmol) at 0 °C and stirred (3 h) to give *N*-[2-(4-chloro-2-methoxyphenoxy)-phenyl]acetamide (85%), and method D was used to convert it to the title compound. Purification by flash chromatography (3% MeOH/$CHCl_3$) gave **19** as a white solid (92%). ^{1}H NMR (400 MHz, CD_3OD): δ = 2.17 (s, 3H), 6.73 (dd, J = 4.0, J = 1 Hz, 1H), 6.84 (dd, J = 4.0, J = 2.0 Hz, 1H), 6.98–6.93 (m, 2H), 7.09–7.03 (m, 2H), 7.76 ppm (dd, J = 3.0, J = 1 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 21.7, 115.1, 116.7, 119.1, 121.7, 122.2, 123.8, 125.1, 127.1, 129.5, 141.5, 149.0, 149.3, 170.3 ppm; HRMS (ESI−): *m/z* calcd for $C_{14}H_{12}ClNO_3$ ($[M-H]^-$): 276.0433, found: 276.0429.

2-(4-Aminophenoxy)-5-chlorophenol (20): Method A was used to prepare intermediate **4a** from 4-chloro-2-methoxyphenol (1.00 g, 6.3 mmol), 1-fluoro-4-nitrobenzene (0.89 g, 6.3 mmol) and K_2CO_3 (1.8 g, 12.6 mmol) in 96% yield. A mixture of **4a** (1.00 g, 3.6 mmol) and 10% Pd/C (0.36 g) in EtOH (20 mL) was stirred at RT under H_2 (4 h). The reaction was filtered through Celite, the filter pad was washed with MeOH and the filtrate was concentrated in vacuo. Purification by flash chromatography (20% EtOAc/hexanes) gave **20a** as a brown liquid (0.82 g, 92%). Method D was used to convert **20a** to the title compound. Purification by flash chromatography (20% EtOAc/hexanes) gave **20** as brown solid (82%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 4.89 (br s, 2H), 6.54 (d, J = 8.8 Hz, 2H), 6.67 (d, J = 8.8 Hz, 2H), 6.74 (dd, J = 4.0, J = 2.0 Hz, 2H), 6.90 ppm (d, J = 2.4 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ = 115.3, 116.9, 119.3, 119.5, 119.7, 119.9, 126.8, 145.2, 145.5, 147.3, 149.5 ppm; HRMS (ESI+): *m/z* calcd for $C_{12}H_{10}ClNO_2$ ($[M+H]^+$): 236.0473, found: 236.0471.

2-(4-Aminophenoxy)-5-propylphenol (21): Method A was used to prepare the intermediate **8a** from 2-methoxy-4-propylphenol (1.00 g, 6.3 mmol), 1-fluoro-4-nitrobenzene (0.89 g, 6.3 mmol) and K_2CO_3 (1.80 g, 12.6 mmol) in 93% yield. A mixture of **8a** (1.00 g,

3.6 mmol) and 10% Pd/C (0.36 g) in MeOH (20 mL) was stirred at RT under H_2 (4 h). The reaction mixture was filtered through Celite, the filter pad was washed with MeOH and the filtrate was concentrated in vacuo. Purification by flash chromatography (20% EtOAc/hexanes) gave **21a** as a brown liquid (0.82 g, 92%). Method D was used to convert **21a** to the title compound. Purification by flash chromatography (3% MeOH/$CHCl_3$) gave **21** as a grey solid (84%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.96$ (t, $J = 7.6$ Hz, 3H), 1.65 (quin, $J = 7.6$ Hz, 2H), 2.53 (t, $J = 7.6$ Hz, 2H), 6.62 (d, $J = 8.0$ Hz, 1H), 6.71–6.67 (m, 3H), 6.89–6.87 ppm (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.4$, 24.1, 37.1, 115.3, 115.9, 116.6, 119.4, 119.8, 138.1, 142.1, 142.5, 146.2, 148.6 ppm; HRMS (ESI−): *m/z* calcd for $C_{15}H_{17}NO_2$ ($[M-H]^-$): 242.1236, found: 242.1238.

4-(4-Chloro-2-hydroxyphenoxy)-1-(4 methylphenylsulphonamido) benzene (22): A solution of **20a** (0.16 g, 0.63 mmol) in anhyd CH_2Cl_2 (3 mL) was cooled to 0 °C and treated with Et_3N (0.2 mL, 1.25 mmol). The reaction was stirred (10 min) and then treated with TsCl (0.18 g, 0.94 mmol), and stirring was continued at RT (3 h). The reaction was quenched with HCl (10 mL, 1 M), diluted with EtOAc (100 mL), the organic layer was separated and the aqueous layer was extracted with EtOAc (×2). The combined organic layers were washed with water then brine, dried (Na_2SO_4), filtered and concentrated in vacuo, and method D was used to convert the crude intermediate to the title compound. Purification by flash chromatography (22% EtOAc/hexanes) gave **22** as a white solid (122 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.41$ (s, 3H), 5.66 (s, 1H), 6.68 (s, 1H), 6.72 (d, $J = 9.0$ Hz, 1H), 6.81 (dd, $J = 9.0$, $J = 2.0$ Hz, 1H), 6.90 (m, 2H), 7.04 (m, 3H), 7.26 (d, $J = 8.0$ Hz, 2H), 7.64 ppm (d, $J = 9.0$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 21.8$, 116.9, 118.9, 119.6, 120.8, 124.7, 127.5, 129.9, 136.2, 142.3, 144.2, 154.5 ppm; MS (ESI−): *m/z* 412 $[M+Na]^+$; HRMS (ESI−): *m/z* calcd for $C_{19}H_{15}ClNO_4S$ ($[M-H]^-$): 388.0416, found 388.0415.

5-Chloro-2-(pyridin-3-yloxy)phenol (23): Method B was used to prepare the intermediate **23a** from 4-chloro-2-methoxyphenol (1 g, 6.3 mmol), KO*t*Bu (0.85 g, 7.6 mmol), $(CuOTf)_2$·PhH (0.33 g, 0.63 mmol) and 3-iodopyridine (1.60 g, 7.6 mmol) in 48% yield, and method D was used to convert the crude intermediate to the title compound. Purification by flash chromatography (3% MeOH/$CHCl_3$) gave **23** as an off-white solid (52%). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 4.83$ (s, 1H), 5.56 (s, 1H), 6.36 (dd, $J = 8.0$, $J = 2.0$ Hz, 1H), 6.58 (d, $J = 2.8$ Hz, 1H), 6.88 (dd, $J = 4.0$, $J = 2.0$ Hz, 1H), 7.03–6.97 (m, 2H), 7.39–7.29 (m, 2H), 8.23 ppm (br s, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 116.7$, 119.3, 122.2, 123.3, 124.1, 130.2, 137.8, 140.6, 141.9, 149.8 ppm; HRMS (ESI−): *m/z* calcd for $C_{11}H_8ClNO_2$ ($[M-H]^-$): 220.0171, found: 220.0170.

***N*-[5-(4-Chloro-2-hydroxyphenoxy)-pyridin-2-yl]acetamide (24)**: Method B was used to prepare the intermediate **24a** from 4-chloro-2-methoxyphenol (1.00 g, 6.3 mmol), *N*-(5-*i*odo-pyridin-2-yl)acetamide (1.4 g, 5.3 mmol), KO*t*Bu (0.78 g, 7.0 mmol) and $(CuOTf)_2$·PhH (0.49 g, 0.95 mmol) in 15% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (8% MeOH/$CHCl_3$) gave **24** as a white solid (60%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.16$ (s, 3H), 6.84 (dd, $J = 4.0$, $J = 2.0$ Hz, 1H), 6.93–6.98 (m, 2H), 7.31(dd, J=8.0, $J = 3.0$ Hz, 1H), 7.98 (d, $J = 2.8$ Hz, 1H), 8.02 ppm (d, $J = 8.9$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 114.9$, 117.0, 119.6, 119.9, 120.7, 121.6, 125.4, 125.9, 126.0, 129.9, 136.6, 136.7, 142.2, 146.6, 148.9, 149.8, 151.1, 170.3 ppm; HRMS (ESI−): *m/z* calcd for $C_{13}H_{11}ClN_2O_3$ ($[M-H]^-$): 277.0385, found: 277.0384.

5-Chloro-2-(pyrazin-2-yloxy)phenol (25): Method B was used to prepare the intermediate **25a** from 4-chloro-2-methoxyphenol (1.00 g, 6.3 mmol), 2-iodopyrazine (1.10 g, 5.3 mmol), KO*t*Bu (0.78 g, 7.0 mmol) and $(CuOTf)_2$·PhH (0.49 g, 0.95 mmol) in 67% yield, and method D was used to convert it to the title compound. Purification by flash chromatography (3% MeOH/$CHCl_3$) gave **25** as a white solid (73%). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 6.88$ (dd, $J = 4.0$, $J = 3.0$ Hz, 1H), 6.96 (d, $J = 2.4$ Hz, 1H), 7.09 (d, $J = 8.8$ Hz, 1H), 8.1 (dd, $J = 2.0$, $J = 1.0$ Hz, 1H), 8.25 (d, $J = 2.8$ Hz, 1H), 8.41 ppm (d, $J = 1.6$ Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 116.4$, 117.2, 118.9, 122.5, 123.3, 125.7, 130.5, 134.4, 137.4, 137.5, 140.6 ppm; HRMS (ESI−): *m/z* calcd for $C_{10}H_7ClN_2O_2$ ($[M-H]^-$): 221.0123, found: 221.0123.

3-(3-Hydroxy-4-phenoxyphenyl)propane-1,2-diol (26): A solution of **27a** (0.10 g, 0.41 mmol) in THF (20 mL) was treated with *N*-methyl-morpholine *N*-oxide (0.20 g, 1.66 mmol) and OsO_4 (0.24 mL, 0.038 mmol, 4 wt.% in H_2O) and stirred until completion (12 h). The reaction was poured into aq Na_2SO_3 (50 mL, 15%) and extracted with CH_2Cl_2. The organic phase was washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Conversion of the crude intermediate using method D gave **26** as a viscous colorless oil without the need for further purification (31 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.75$ (t, $J = 7.7$ Hz, 2H), 3.58–3.54 (m, 1H), 3.75–3.72 (m, 1H), 3.97 (br s, 1H), 5.47 (s, 1H), 6.68 (dd, $J = 8.0$, $J = 1.0$ Hz, 1H), 6.82 (d, $J = 8.1$ Hz, 1H), 5.66 (s, 1H), 6.72 (dd, $J = 8.0$, $J = 1.0$ Hz, 1H), 6.85 (d, $J = 8.2$ Hz, 1H), 6.95 (s, 1H), 7.04 (d, $J = 8.0$ Hz, 2H), 7.14 (t, $J = 7.3$ Hz, 1H), 7.36 ppm (t, $J = 7.8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 39.2$, 66.0, 72.8, 116.8, 117.8, 118.9, 121.3, 123.6, 129.8, 134.4, 142.1, 147.4 ppm; HRMS (ESI−): *m/z* calcd for $C_{15}H_{16}O_4$ ($[M-H]^-$): 259.0975, found: 259.0978.

2-Phenoxy-5-propylphenol (27): A suspension of **27a** (0.10 g, 0.41 mmol) and 10% Pd/C (0.02 g) in EtOAc (5 mL) was stirred under H_2 at RT (4 h). The reaction was filtered through Celite, the filter pad was washed with EtOAc, and the filtrate was concentrated in vacuo. Method D was used to convert the crude intermediate to the title compound. Purification by preparative TLC (30% EtOAc/hexanes) gave **27** as an off-white solid (54 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.97$ (t, $J = 7.3$ Hz, 3H), 1.69–1.62 (m, 2H), 2.56 (t, $J = 7.5$ Hz, 2H), 5.49 (s, 1H), 6.68 (dd, $J = 8.0$, $J = 1.0$ Hz, 1H), 6.83 (d, $J = 8.2$ Hz, 1H), 6.90 (d, $J = 1.6$ Hz, 1H), 7.03 (d, $J = 7.8$ Hz, 2H), 7.12 (t, $J = 7.3$ Hz, 1H), 7.35 ppm (t, $J = 7.7$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.7$, 24.4, 37.5, 116.0, 117.5, 118.9, 120.5, 123.2, 129.7, 139.8, 141.0, 147.2, 157.1 ppm; MS (ESI−): *m/z* 227 $[M-H]^-$; HRMS (ESI+): *m/z* calcd for $C_{15}H_{16}O_2$ ($[M+H]^+$): 229.1223, found 229.1224.

5-Hydroxymethyl-2-phenoxyphenol (28): Method D was used to prepare the title compound from **28a** (0.08 g, 0.34 mmol) and BBr_3 (1 M, 6.9 mL, 6.9 mmol). Purification by preparative TLC (40% EtOAc/hexanes) gave **28** as a colorless oil (64 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.48$ (s, 2H), 5.66 (s, 1H), 6.82 (d, $J = 7.3$ Hz, 1H), 6.89 (dd, $J = 8.0$, $J = 2.0$ Hz, 1H), 7.06 (d, $J = 8.3$ Hz, 2H), 7.12 (d, $J = 2.0$ Hz, 1H), 7.17 (t, $J = 7.3$ Hz, 1H), 7.38 ppm (t, $J = 7.8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 33.2$, 116.7, 118.2, 118.4, 121.3, 124.0, 129.9, 134.0, 143.9, 147.2, 156.1 ppm; MS (ESI+): *m/z* 239 $[M+Na]^+$.

5-[(1-Methyl-butylamino)methyl]-2-phenoxyphenol (29): Method D was used to prepare the title compound from **29a** (0.10 g, 0.33 mmol) and BBr_3 (1 M, 6.6 mL, 6.6 mmol). Purification by preparative TLC (10% MeOH/CH_2Cl_2) gave **29** as a colorless oil (65 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.91$ (t, $J = 7.4$ Hz, 3H), 1.09 (d, $J = 11.0$ Hz, 3H), 1.41–1.34 (m, 1H), 1.58–1.51 (m, 1H), 2.67–2.62 (m, 1H), 3.78–3.67 (m, 2H), 6.79–6.77 (m, 1H), 6.84 (d, $J = 8.1$ Hz, 1H), 6.99 (d, $J = 7.9$ Hz, 2H), 7.01 (d, $J = 1.4$ Hz, 1H), 7.10 (d, $J = 7.3$ Hz,

1H), 7.33 ppm (t, $J=7.8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 10.2, 19.5, 29.6, 50.7, 53.9, 116.6, 117.5, 119.2, 120.1, 123.1, 129.7, 137.4, 142.3, 147.7, 157.1, 165.0 ppm.

2-Phenoxy-5-piperidin-1-ylmethylphenol (30): Method D was used to prepare the title compound from **30a** (0.07 g, 0.23 mmol) and BBr_3 (4.7 mL, 1 M in CH_2Cl_2). Purification by preparative TLC (1:10 MeOH/CH_2Cl_2) gave **30** as a grey solid (34 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=1.47$ (br s, 2H), 1.70–1.60 (m, 4H), 2.47 (br s, 4H), 3.49 (s, 2H), 6.83 (s, 2H), 7.03 (d, $J=7.8$ Hz, 2H), 7.12 (t, $J=$ 7.3 Hz, 1H), 7.07 (s, 1H), 7.35 ppm (t, $J=8.0$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=24.1$, 25.5, 54.2, 63.0, 117.2, 117.8, 118.6, 121.4, 123.4, 129.8, 142.5, 147.3, 156.9 ppm; HRMS (ESI+): *m/z* calcd for $C_{18}H_{21}NO_2$ ($[M+H]^+$): 284.1645, found 284.1652.

5-(Benzylaminomethyl)-2-phenoxyphenol (31): Method D was used to prepare the title compound from **31a** (0.08 g, 0.25 mmol) and BBr_3 (1 M, 5 mL, 5.0 mmol). Purification by preparative TLC (10% MeOH/CH_2Cl_2) gave **31** as a white solid (42 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=3.78$ (s, 2H), 3.86 (s, 2H), 6.84 (s, 2H), 7.00 (d, $J=8.0$ Hz, 2H), 7.14–7.10 (m, 2H), 7.42–7.28 ppm (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=51.5$, 52.0, 116.6, 117.8, 119.0, 120.6, 123.4, 127.5, 128.5, 128.6, 128.7, 129.8, 134.4, 137.6, 142.9, 147.7, 156.8 ppm. HRMS (ESI−): *m/z* calcd for $C_{20}H_{19}NO_2$ ($[M-H]^-$): 304.1343, found: 304.1352.

2-[4-(2-Hydroxy-4-chlorophenoxy)phenoxy]-5-chlorophenol (32): Method C was used to prepare intermediate **32a** from 2-methoxy-4-propylphenol (0.20 g, 1.2 mmol) and benzene-1, 4-diboronic acid (0.20 g, 1.2 mmol). Purified by preparative TLC (30% EtOAc/hexanes) gave **32a** (35 mg, 7%). Method D was used to prepare the title compound from **32a** (0.03 g, 0.076 mmol) and BBr_3 (1 M, 1.5 mL, 1.5 mmol). Purification by preparative TLC (40% EtOAc/hexanes) gave **32** as a grey solid (12 mg, 43%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$): $\delta=6.86$–6.83 (m, 6H), 6.93 (d, $J=7.5$ Hz, 2H), 6.98 (d, $J=$ 2.3 Hz, 2H), 10.06 ppm (s, 2H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$): $\delta=$ 117.3, 118.3, 119.7, 122.6, 128.5, 143.1, 150.4, 152.9 ppm. HRMS (ESI−): *m/z* calcd for $C_{18}H_{12}Cl_2O_4$ ($[M-H]^-$): 361.0040, found: 361.0047.

2-[3-(2-Hydroxy-4-chlorophenoxy)phenoxy]-5-chlorophenol (33): Method C was used to prepare intermediate **33a** from 2-methoxy-4-propylphenol (0.20 g, 1.20 mmol), and benzene-1, 3-diboronic acid (0.20 g, 1.20 mmol). Purification by preparative TLC (30% EtOAc/hexanes) gave **33a** (207 mg, 41%). Method D was used to prepare the title compound from **33a** (0.1 g, 0.25 mmol) and BBr_3 (1 M, 5.1 mL, 5.1 mmol). Purification by preparative TLC (40% EtOAc/hexanes) gave **33** as a white solid (65 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.96$ (t, $J=7.3$ Hz, 6H), 1.70–1.60 (m, 4H), 2.55 (t, $J=7.4$ Hz, 4H), 5.68 (s, 2H), 6.76–6.71 (m, 3H), 6.86 (s, 4H), 7.08 (t, $J=1.2$ Hz, 2H), 7.30 ppm (t, $J=8.2$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=107.2$, 111.9, 112.2, 116.4, 116.5, 119.1, 119.7, 120.4, 129.7, 130.5, 141.1, 147.7, 157.6 ppm. HRMS (ESI−): *m/z* calcd for $C_{18}H_{12}Cl_2O_4$ ($[M-H]^-$): 361.0040, found: 361.0051.

2-[3-(2-Hydroxy-4-propylphenoxy)phenoxy]-5-propylphenol (34): Method C was used to prepare intermediate **34a** from 2-methoxy-4-propylphenol (0.20 g, 1.2 mmol) and benzene-1,3-diboronic acid (0.20 g, 1.2 mmol). Purification by preparative TLC (30% EtOAc/hexanes) gave **34a** (27 mg, 5%). Method D was used to prepare the title compound from **34a** (0.10 g, 0.24 mmol) and BBr_3 (1 M, 4.9 mL, 4.9 mmol). Purification by preparative TLC (40% EtOAc/hexanes) gave **34** as a viscous oil (67 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.97$ (t, $J=7.3$ Hz, 6H), 1.70–1.60 (m, 4H), 2.56 (t, $J=$ 7.4 Hz, 4H), 5.40 (s, 2H), 6.75–6.65 (m, 5H), 6.86 (s, 1H), 6.88 (t, $J=$ 1.8 Hz, 3H), 7.27–7.22 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 13.3, 24.0, 37.1, 106.4, 111.3, 115.9, 119.0, 120.3, 130.1, 139.9, 146.8, 158.2 ppm; HRMS (ESI−): *m/z* calcd for $C_{24}H_{26}O_4$ ($[M-H]^-$): 377.1758, found: 377.1763.

2-[4-(2-Hydroxy-4-propylphenoxy)phenoxy]-5-propylphenol (35): Method C was used to prepare intermediate **35a** from 2-methoxy-4-propylphenol (0.20 g, 1.2 mmol) and benzene-1,4-diboronic acid (0.20 g, 1.2 mmol). Purification by preparative TLC (30% EtOAc/hexanes) gave **35a** (27 mg, 5%). Method D was used to prepare the title compound from **35a** (0.02 g, 0.049 mmol) and BBr_3 (1 M, 0.98 mL, 0.98 mmol). Purification by preparative TLC (40% EtOAc/hexanes) gave **35** as a viscous oil (13 mg, 69%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.96$ (t, $J=7.3$ Hz, 6H), 1.70–1.60 (m, 4H), 2.55 (t, $J=$ 7.4 Hz, 4H), 5.51 (s, 2H), 6.67 (dd, $J=8.0$, $J=2.0$ Hz, 2H), 6.79 (d, $J=8.2$ Hz, 2H), 6.89 (d, $J=1.8$ Hz, 2H), 7.00 ppm (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=13.3$, 24.0, 37.1, 115.6, 117.8, 118.7, 120.1, 139.2, 141.1, 146.5, 152.3 ppm; HRMS (ESI−): *m/z* calcd for $C_{24}H_{26}O_4$ ($[M-H]^-$): 377.1758, found: 377.1747.

2-(Biphenyl-3-yloxy)-5-propylphenol (36): Method C was used to prepare intermediate **36a** from 4-propyl-2-methoxyphenol (0.10 g, 0.6 mmol) and 3-biphenylboronic acid (0.36 g, 1.8 mmol) in 46% yield. Method D was used to prepare the title compound from **36a** (0.08 g, 0.25 mmol) and BBr_3 (1 M, 5.0 mL, 5.0 mmol). Purification by preparative TLC (40% EtOAc/hexanes) gave **36** as a white solid (34 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.99$ (t, $J=7.3$ Hz, 3H), 1.75–1.65 (m, 2H), 2.58 (t, $J=7.4$ Hz, 2H), 5.56 (s, 1H), 6.71 (d, $J=8.2$ Hz, 1H), 6.90 (d, $J=8.2$ Hz, 1H), 6.93 (s, 1H), 7.01 (d, $J=$ 6.7 Hz, 1H), 7.42–7.35 (m, 3H), 7.46 (t, $J=7.3$ Hz, 2H), 7.59 ppm (d, $J=7.4$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=13.4$, 24.0, 37.1, 115.7, 115.9, 116.0, 118.6, 120.2, 121.7, 126.7, 127.2, 128.4, 129.7, 139.5, 139.9, 140.6, 142.8, 146.8, 157.2 ppm; HRMS (ESI+): *m/z* calcd for $C_{29}H_{34}N_4O_5$ ($[M+H]^+$): 519.2602, found: 519.2595.

3-(2-Hydroxy-4-propylphenoxy)benzoic acid methyl ester (37): Method C was used to prepare intermediate **37a** from 4-propyl-2-methoxyphenol (0.10 g, 0.6 mmol) and 3-(ethoxycarbonyl) phenylboronic acid (0.32 g, 1.8 mmol) in 41% yield. Method D was used to prepare the title compound from **37a** (0.07 g, 0.23 mmol) and BBr_3 (1 M, 4.6 mL, 4.6 mmol). Purification by preparative TLC (50% EtOAc/hexanes) gave **37** as a white solid (44 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.97$ (t, $J=7.3$ Hz, 3H), 1.69–1.62 (m, 2H), 2.57 (t, $J=7.5$ Hz, 2H), 3.91 (s, 3H), 5.56 (s, 1H), 6.72 (dd, $J=8.0$, $J=2.0$ Hz, 1H), 6.82 (d, $J=8.2$ Hz, 1H), 6.91 (d, $J=1.8$ Hz, 1H), 7.22–7.19 (m, 1H), 7.40 (t, $J=8.0$ Hz, 1H), 7.68–7.67 (m, 1H), 7.77 ppm (d, $J=7.7$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=13.7$, 24.4, 37.5, 52.2, 116.4, 118.4, 119.0, 120.7, 121.9, 124.3, 129.8, 131.9, 140.3, 140.6, 147.2, 157.3, 166.4 ppm; HRMS (ESI−): *m/z* calcd for $C_{17}H_{18}O_4$ ($[M-H]^-$): 285.1132, found: 285.1139.

2-(3-Hydroxymethyl-phenoxy)-5-propylphenol (38): Method C was used to prepare intermediate **38a** from 4-propyl-2-methoxyphenol (0.10 g, 0.6 mmol) and 3-(hydroxymethyl) phenylboronic acid (0.27 g, 1.8 mmol) in 18% yield. Method D was used to prepare the title compound from **38a** (0.03 g, 0.11 mmol) and BBr_3 (1 M, 2.2 mL, 2.2 mmol). Purification by preparative TLC (60% EtOAc/hexanes) gave **38** as an oil (12 mg, 42%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=0.98$ (t, $J=7.3$ Hz, 3H), 1.78–1.62 (m, 2H), 2.57 (t, $J=$ 7.4 Hz, 2H), 5.45 (s, 1H), 6.70 (dd, $J=8.0$, $J=2.0$ Hz, 1H), 6.84 (d, $J=8.2$ Hz, 1H), 6.91 (d, $J=1.8$ Hz, 1H), 6.94 (dd, $J=8.0$, $J=2.0$ Hz, 1H), 7.06 (d, $J=1.9$ Hz, 1H), 7.14 (d, $J=7.6$ Hz, 1H), 7.29 ppm (d, $J=8.8$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=13.8$, 24.4, 32.7, 37.5, 116.2, 117.3, 117.9, 119.1, 120.7, 123.8, 125.8, 130.1, 139.7, 140.2, 140.5, 147.2, 157.4 ppm; MS (ESI−): *m/z* 257 $[M-H]^-$.

3-(2-Hydroxy-4-propylphenoxy)benzoic acid (39): A solution of **37** (0.02 g, 0.073 mmol) in MeOH/H_2O (10 mL, 1:1) was treated with LiOH·H_2O (0.03 g, 0.73 mmol) and stirred at RT (2 h). The reaction was acidified with saturated aq $KHSO_4$ and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by preparative TLC (1:10 MeOH/CH_2Cl_2) gave **39** as a white solid (11 mg, 55%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.98$ (t, $J = 7.3$ Hz, 3H), 1.70–1.60 (m, 2H), 2.57 (t, $J = 7.5$ Hz, 2H), 6.71 (dd, $J = 8$, $J = 1$ Hz, 1H), 6.84 (d, $J = 8.2$ Hz, 1H), 6.92 (d, $J = 1.5$ Hz, 1H), 7.27 (dd, $J = 8$, $J = 2$ Hz, 1H), 7.45 (t, $J = 8.0$ Hz, 1H), 7.74 (s, 1H), 7.86 ppm (d, $J = 7.6$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.7$, 24.4, 37.5, 116.4, 118.8, 119.1, 120.8, 122.8, 124.9, 129.9, 130.2, 131.0, 140.4, 147.2, 157.4, 171.2 ppm; HRMS (ESI−): *m/z* calcd for $C_{16}H_{16}O_4$ ($[M-H]^-$): 271.0975, found: 271.0982.

1-[4-(2-Hydroxy-4-propylphenoxy)phenyl]ethanone (40): Method C was used to prepare intermediate **40a** from 2-methoxy-4-propylphenol (0.10 g, 0.6 mmol) and 4-acetylphenylboronic acid (0.30 g, 1.8 mmol) in 39% yield. Method D was used to prepare the title compound from **40a** (0.06 g, 0.21 mmol) and BBr_3 (1 M, 4.2 mL, 4.2 mmol). Purification by preparative TLC (40% EtOAc/hexanes) gave **40** as a grey solid (42 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.98$ (t, $J = 7.2$ Hz, 3H), 1.75–1.62 (m, 2H), 2.60–2.50 (m, 5H), 5.37 (s, 1H), 6.74 (d, $J = 8.0$ Hz, 1H), 6.90 (d, $J = 8.5$ Hz, 1H), 6.92 (s, 1H), 7.05 (d, $J = 8.5$ Hz, 2H), 7.96 ppm (d, $J = 8.5$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.7$, 24.4, 26.4, 37.5, 116.4, 116.6, 120.1, 120.9, 130.6, 132.2, 139.5, 141.1, 147.4, 161.5, 196.6 ppm; HRMS (ESI−): *m/z* calcd for $C_{17}H_{18}O_3$ ($[M-H]^-$): 269.1183, found: 269.1188.

2-(4-Methylsulfanylphenoxy)-5-propylphenol (41): Method D was used to prepare the title compound from **41a** (0.05 g, 0.17 mmol) and BBr_3 (1 M, 3.4 mL, 3.4 mmol). Purification by preparative TLC (40% EtOAc/hexanes) gave **41** as a grey solid (32 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.97$ (t, $J = 7.2$ Hz, 3H), 1.72–1.62 (m, 2H), 2.49 (s, 3H), 2.56 (t, $J = 6.4$ Hz, 2H), 5.47 (s, 1H), 6.67 (d, $J = 8.2$ Hz, 1H), 6.80 (d, $J = 8.2$ Hz, 1H), 6.89 (s, 1H), 6.97 (d, $J = 8.7$ Hz, 2H), 7.29–7.27 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.7$, 17.1, 24.4, 37.5, 116.1, 118.3, 118.6, 120.5, 125.8, 129.2, 132.3, 139.8, 141.1, 147.0, 155.2 ppm; HRMS (ESI−): *m/z* calcd for $C_{16}H_{18}O_2S$ ($[M-H]^-$): 273.0954, found: 273.0958.

2-[4-(1-Hydroxyethyl)phenoxy]-5-propylphenol (42): $NaBH_4$ (0.008 g, 0.22 mmol) was added in a single portion to a solution of **40** (0.02 g, 0.073 mmol) in anhyd MeOH (5 mL) at 0 °C and the reaction was stirred for 1 h. After quenching with H_2O (2 mL), the reaction was diluted with EtOAc (15 mL). The organic phase was washed with brine, dried ($NaSO_4$), filtered and concentrated in vacuo. Purification by preparative TLC (50% EtOAc/hexanes) gave **42** as a clear oil (14 mg, 69%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.96$ (t, $J = 7.3$ Hz, 3H), 1.70–1.55 (m, 2H), 2.56 (t, $J = 7.4$ Hz, 2H), 4.91 (q, $J = 6.4$ Hz, 1H), 5.50 (s, 1H), 6.67 (dd, $J = 8$, $J = 2$ Hz, 1H), 6.82 (d, $J = 8.2$ Hz, 1H), 6.89 (d, $J = 1.7$ Hz, 1H), 7.00 (d, $J = 8.2$ Hz, 2H), 7.35 ppm (d, $J = 8.5$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.7$, 24.4, 25.1, 37.5, 69.8, 116.1, 117.5, 118.9, 120.5, 126.9, 139.8, 140.7, 141.0, 147.1, 156.5 ppm; HRMS (ESI−): *m/z* calcd for $C_{17}H_{20}O_3$ ($[M-H]^-$): 271.1339, found: 271.1342.

2-(4-Methanesulfinylphenoxy)-5-propylphenol (43): Method C was used to prepare intermediate **41a** from 2-methoxy-4-propylphenol (0.20 g, 1.2 mmol) and 4-(methylsulfanyl) phenylboronic acid (0.61 g, 3.61 mmol) in 59% yield. A solution of **41a** (0.15 g, 0.52 mmol) in CH_2Cl_2 (20 mL) was cooled to 0 °C and treated with a solution of *m*-CPBA (80%, 0.17 g, 0.78 mmol) in CH_2Cl_2 (3 mL) to give a mixture of **43a** (35 mg, 22%) and **44a** (54 mg, 32%). Method D was used to prepare the title compound from **43a** (0.03 g, 0.098 mmol) and BBr_3 (1 M, 1.9 mL, 1.9 mmol). Purification by preparative TLC (60% EtOAc/hexanes) gave **43** as a grey solid (19 mg, 66%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.97$ (t, $J = 7.1$ Hz, 3H), 1.70–1.58 (m, 2H), 2.57 (t, $J = 6.7$ Hz, 2H), 2.73 (s, 3H), 5.86 (s, 1H), 6.72 (d, $J = 8.0$ Hz, 1H), 6.88 (d, $J = 8.1$ Hz, 1H), 6.92 (s, 1H), 7.12 (d, $J = 6.9$ Hz, 2H), 7.60 ppm (d, $J = 6.9$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.7$, 24.4, 37.5, 43.9, 116.8, 117.6, 120.1, 120.8, 125.5, 138.9, 139.8, 141.0, 147.5, 160.1 ppm; HRMS (ESI−): *m/z* calcd for $C_{16}H_{18}O_3S$ ($[M-H]^-$): 289.0903, found: 289.0910.

2-(4-Methanesulfonylphenoxy)-5-propylphenol (44): Method D was used to prepare the title compound from **44a** (0.05 g, 0.15 mmol) and BBr_3 (1 M, 3.1 mL, 3.1 mmol). Purification by preparative TLC (60% EtOAc in hexane) gave **44** as a white solid (24 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.98$ (t, $J = 7.2$ Hz, 3H), 1.75–1.65 (m, 2H), 2.59 (t, $J = 6.6$ Hz, 2H), 3.06 (s, 3H), 5.41 (s, 1H), 6.76 (d, $J = 8$ Hz, 1H), 6.91 (d, $J = 8$ Hz, 1H), 6.93 (s, 1H), 7.12 (d, $J = 6.7$ Hz, 2H), 7.89 ppm (d, $J = 6.6$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.7$, 24.3, 37.5, 44.7, 116.9, 117.0, 120.4, 121.1, 129.7, 134.4, 139.1, 141.6, 147.4, 162.0 ppm; HRMS (ESI−): *m/z* calcd for $C_{16}H_{18}O_4S$ ($[M-H]^-$): 305.0853, found: 305.0858.

2-(3-Dimethylaminophenoxy)-5-propylphenol (45): Method D was used to prepare the title compound from **45b** (0.09 g, 0.31 mmol) and BBr_3 (1 M, 6.3 mL, 6.3 mmol). Purification by flash chromatography (40% EtOAc/hexanes) gave **45** as an off-white solid (70 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.97$ (t, $J = 7.3$ Hz, 3H), 1.68–1.63 (m, 2H), 2.56 (t, $J = 7.5$ Hz, 2H), 2.95 (s, 6H), 5.52 (s, 1H), 6.32 (d, $J = 7.6$ Hz, 1H), 6.44 (s, 1H), 6.49 (d, $J = 8.3$ Hz, 1H), 6.66 (d, $J = 7.1$ Hz, 1H), 6.85 (d, $J = 8.2$ Hz, 1H), 6.86 (s, 1H), 7.18 ppm (t, $J = 8.1$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.4$, 24.0, 37.1, 40.0, 101.7, 104.8, 107.2, 115.4, 118.4, 120.2, 129.6, 139.0, 140.9, 146.7, 151.6, 157.7 ppm; HRMS (ESI−): *m/z* calcd for $C_{17}H_{21}NO_2$ ($[M-H]^-$): 270.1499, found: 270.1501.

5-Propyl-2-(3-trifluoromethylphenoxy)phenol (46): Method D was used to prepare the title compound from **46b** (0.08 g, 0.25 mmol) and BBr_3 (1 M, 5.1 mL, 5.1 mmol). Purification by preparative TLC (40% EtOAc/hexanes) gave **46** as a grey solid (45 mg, 58%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.98$ (t, $J = 7.3$ Hz, 3H), 1.72–1.62 (m, 2H), 2.58 (t, $J = 6.8$ Hz, 2H), 5.40 (s, 1H), 6.72 (d, $J = 8.1$ Hz, 1H), 6.85 (d, $J = 8.1$ Hz, 1H), 6.92 (s, 1H), 7.17 (d, $J = 8.1$ Hz, 1H), 7.37 (d, $J = 7.3$ Hz, 1H), 7.45 ppm (t, $J = 7.9$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.3$, 24.0, 37.1, 113.9, 113.9, 116.1, 118.9, 119.4, 119.4, 119.9, 120.5, 121.8, 124.5, 130.0, 131.4, 131.7, 132.0, 139.7, 140.4, 146.8, 157.2 ppm; HRMS (ESI−): *m/z* calcd for $C_{16}H_{19}F_3O_2$ ($[M-H]^-$): 295.0951, found: 295.0973.

3-(2-Hydroxy-4-propylphenoxy)benzaldehyde (47): Method D was used to prepare the title compound from **47a** (0.25 g, 0.92 mmol) and BBr_3 (1 M, 18.4 mL, 18.4 mmol). Purification by flash chromatography (60% EtOAc/hexanes) gave **47** (187 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.98$ (t, $J = 7.2$ Hz, 3H), 1.70–1.63 (m, 2H), 2.57 (t, $J = 6.7$ Hz, 2H), 6.71 (dd, $J = 8$, $J = 2$ Hz, 1H), 6.85 (d, $J = 8.3$ Hz, 1H), 6.97 (d, $J = 1.7$ Hz, 1H), 7.32–7.29 (m, 1H), 7.49 (s, 1H), 7.53 (d, $J = 7.9$ Hz, 1H), 7.63 (d, $J = 7.3$ Hz, 1H), 9.98 ppm (s, 1H).

3-[3-(2-Hydroxy-4-propylphenoxy)phenyl]acrylic acid methyl ester (48): A solution of **47** (0.18 g, 0.70 mmol) and methyl (triphenylphosphoranylidene) acetate (0.47 g, 1.40 mmol) in THF (20 mL) was heated at reflux overnight, cooled and concentrated in vacuo. Purification by flash chromatography (1:3 EtOAc/hexanes) gave **48** as a white solid (177 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.98$ (t, $J = 7.3$ Hz, 3H), 1.72–1.60 (m, 2H), 2.57 (t, $J = 7.0$ Hz, 2H), 3.82 (s,

3H), 5.43 (s, 1H), 6.40 (d, J=16.0 Hz, 1H), 6.70 (d, J=8.1 Hz, 1H), 6.84 (d, J=8.1 Hz, 1H), 6.91 (s, 1H), 7.05 (d, J=8.3 Hz, 1H), 7.16 (s, 1H), 7.36 (t, J=7.8 Hz, 1H), 7.64 ppm (d, J=16.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.7, 24.4, 37.5, 51.7, 116.3, 116.4, 118.7, 119.2, 120.7, 123.0, 130.2, 136.2, 140.3, 140.4, 144.0, 147.2, 157.7, 167.1 ppm; HRMS (ESI−): *m/z* calcd for $C_{19}H_{20}O_4$ ([M−H]$^-$): 311.1288, found: 311.1299.

3-[3-(2-Hydroxy-4-propylphenoxy)phenyl]propionic acid methyl ester (49): A suspension of **48** (0.10 g, 0.32 mmol) and 10% Pd/C (0.02 g) in EtOAc (10 mL) was stirred under H_2 (2 h), filtered over Celite and concentrated in vacuo to give **49** as a colorless oil (88 mg, 87%) without the need for further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.97 (t, J=7.3 Hz, 3H), 1.69–1.62 (m, 2H), 1.64 (t, J=7.8 Hz, 1H), 2.56 (t, J=7.4 Hz, 2H), 2.63 (t, J=7.9 Hz, 2H), 2.94 (t, J=7.6 Hz, 2H), 5.47 (s, 1H), 3.68 (s, 3H), 6.68 (dd, J=8, J=2 Hz, 1H), 6.82 (d, J=8.1 Hz, 1H), 6.87–6.85 (m, 2H), 6.89 (d, J=1.5 Hz, 1H), 6.95 (d, J=7.4 Hz, 1H), 7.25 ppm (t, J=7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.7, 24.4, 30.7, 35.4, 37.5, 51.6, 115.3, 116.0, 117.3, 118.9, 120.5, 123.1, 129.8, 139.8, 140.9, 142.6, 147.2, 157.3, 173.1 ppm; HRMS (ESI−): *m/z* calcd for $C_{19}H_{22}O_4$ ([M−H]$^-$): 313.1445, found: 313.1451.

3-[3-(2-Hydroxy-4-propylphenoxy)phenyl]propionic acid (50): A solution of **48** (0.05 g, 0.16 mmol) in MeOH (10 mL) was treated with LiOH·H_2O (0.07 g, 1.62 mmol) and stirred until completion (2 h) to give **50** as a white solid (29 mg, 60%) without the need for further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.98 (t, J=7.3 Hz, 6H), 1.70–1.60 (m, 4H), 2.58 (t, J=7.4 Hz, 4H), 6.41 (d, J=16.0 Hz, 1H), 6.71 (d, J=8.0 Hz, 1H), 6.85 (d, J=8.0 Hz, 1H), 6.92 (s, 1H), 7.07 (d, J=8.0 Hz, 1H), 7.18 (s, 1H), 7.29 (d, J=8.0 Hz, 1H), 7.38 (t, J=7.6 Hz, 1H), 7.62 ppm (d, J=16.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.8, 24.4, 37.5, 116.4, 116.6, 118.1, 119.2, 119.6, 120.8, 123.2, 130.3, 135.8, 140.3, 140.4, 146.2, 147.2, 157.8, 171.7 ppm; HRMS (ESI−): *m/z* calcd for $C_{18}H_{18}O_4$ ([M−H]$^-$): 297.1132, found: 297.1137.

3-[3-(2-Hydroxy-4-propylphenoxy)phenyl]propionic acid (51): A solution of **49** (0.05 g, 0.16 mmol) in MeOH (10 mL) was treated with LiOH·H_2O (0.07 g, 1.62 mmol) and stirred until completion (2 h) to give **50** as a white solid (37 mg, 75%) without the need for further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.97 (t, J=7.3 Hz, 3H), 1.72–1.60 (m, 2H), 2.56 (t, J=7.4 Hz, 2H), 2.68 (t, J=7.8 Hz, 2H), 2.95 (t, J=7.5 Hz, 2H), 6.67 (dd, J=8, J=2 Hz, 1H), 6.82 (d, J=8.2 Hz, 1H), 6.92–6.85 (m, 3H), 6.93 (d, J=7.4 Hz, 1H), 7.24 ppm (d, J=7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.7, 24.4, 30.4, 35.1, 37.5, 115.4, 116.1, 117.4, 119.0, 120.5, 123.1, 129.8, 139.9, 140.8, 142.2, 147.2, 157.3, 177.7 ppm; HRMS (ESI−): *m/z* calcd for $C_{18}H_{20}O_4$ ([M−H]$^-$): 299.1288, found: 299.1298.

3-[5-Chloro-2-(2,4-dichlorophenoxy)phenoxy]pyridine (52): Method C was used to prepare the title compound from triclosan (0.10 g, 0.34 mmol), 3-pyridineboronic acid (0.13 g, 1.03 mmol), $Cu(OAc)_2$ (0.31 g, 1.72 mmol), Et_3N (0.35 g, 3.45 mmol) and 5 Å molecular sieves (0.6 g) in CH_2Cl_2 (20 mL). Purification by preparative TLC (1:3 EtOAc/hexanes) gave **52** as a grey solid (45 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.83 (d, J=8.7 Hz, 1H), 6.94 (d, J=8.6 Hz, 1H), 7.20–7.13 (m, 3H), 7.27–7.26 (m, 2H), 7.42 (d, J=2.4 Hz, 1H), 8.33 (s, 1H), 8.39 ppm (d, J=2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=119.5, 121.1, 122.0, 124.0, 124.4, 125.5, 125.6, 127.9, 129.3, 129.9, 130.5, 140.2, 144.7, 145.8, 146.5, 150.8, 153.2 ppm; HRMS (ESI−): *m/z* calcd for $C_{17}H_{11}O_2NCl_3$ ([M−H]$^-$): 365.9849, found: 365.9843.

X-ray crystallography

Details on the crystal structure of the triclosan-bound complex have been reported elsewhere.[46] The atomic coordinates and structure factors have been deposited in the Protein Data Bank (http://www.rcsb.org/pdb) under the PDB code 2QIO. A summary of crystallographic data collection and refinement information for inhibitors **11** and **43** bound to *Ba*ENR is provided in the Supporting Information (unpublished results).

Computational methods

Compounds were modeled into our triclosan·*Ba*ENR crystal structure using the GOLD docking program.[58] The triclosan crystal structure was used as a reference ligand, and default parameters were chosen. After testing different algorithms, ChemScore was selected from within Gold as the best scoring function. A CoMFA was performed using up to 10 docking conformations for each of our ligands. The best scoring conformation was initially selected, then alternative conformations were systematically replaced to determine whether a better alignment could be achieved over the initial one, by way of a cross-validated PLS analysis. After all the conformations were evaluated, the conformation associated with the highest q^2 was selected. Using a training set of 22 compounds, an acceptable model was developed, shown by the PLS results of q^2=0.831, r^2=0.929, and SEE=0.36. The steric and electrostatic fields were graphed, and used in the design of new compounds.

Purification of *Ba*ENR and activity assays

*Ba*ENR was expressed in, and purified from *E. coli* according to the procedures of Klein et al.[46] The assay was conducted at 30 °C in a 96-well plate using purified *Ba*ENR (1 μM), NADH (175 μM), and crotonyl-CoA (200 μM) with 200 μL of buffer containing Tris-HCl (20 mM), NaCl (150 mM). Crotonyl-CoA was added last to initiate the reaction. *Ba*ENR activity, determined by the decrease in NADH absorbance at 340 nm (ε=6220 M^{-1}cm^{-1}), was recorded using a Molecular Devices SpectraMax384 Plus plate reader. The path length of the well in the 96-well microtiter plate cell was determined by measuring the rate (ΔAU/min) in a 1 mL reaction volume with a 1.00 cm cuvette, and then calculating the rate of reaction (NADH/min) using the Beer–Lambert equation (AU=ε·l·[NADH]) where l=path length. The reaction was then alloquoted (200 μL) into 5 wells of the 96-well plate, and the reaction rates were measured (ΔAU/min). Values for ΔAU/min ([NADH] min^{-1}), and ε (6220 M^{-1}cm^{-1}) allowed us to determine the path length for the reactions (0.45 cm).

Inhibition of *Ba*ENR by triclosan and other compounds

The maximum solubility of a compound was determined by adding a stock solution of the compound in DMSO to the reaction well (2 μL, 10 mM) with a final volume of 200 μL; if precipitation was visible in the reaction well, sequential twofold dilutions of the stock solution in DMSO were carried out until precipitation in the reaction well was no longer visible. This protocol ensured a consistent 1% (*v/v*) of DMSO in the final solution. The percent inhibition (%I) was tested at the maximum solubility concentration using the equation %I=((A_C−A_I)/A_C)100, where A_C=activity of the control (uninhibited) and A_I=activity in the presence of an inhibitor. If %I ≥50%, then the IC_{50} value was determined by fixing the non-varied substrates at subsaturating concentrations as described above, and then varying the inhibitor concentration. The reactions

were allowed to preincubate for 30 min before initiation with crotonyl-CoA. An equal concentration of DMSO was added to the control experiments to negate the effect of solvent on enzyme activity. Nonlinear regression and the Enzyme Kinetics Module 1.0 of the Sigma Plot (SYSTAT Inc) program were used to fit the data to the equation $\%I = \%I_{max}/(IC_{50}/[I] + 1)$.

Bacterial growth and MIC determination

The MIC values were determined against the ΔANR (plasmid-cured Ames Strain) and Sterne strains of *B. anthracis*, and reference strains of the following bacteria: *S. aureus* (ATCC strain **29213**), MRSA (ATCC strain **43300**), *Enterococcus fecalis* (ATCC strain **29212**), vancomycin-resistant *Enterococcus* (VRE) (ATCC strain **51299**), *Listeria monocytogenes* (**10403 S**),[59] *P. aeruginosa* (ATCC strain **27853**), *Klebsiella pneumoniae* (ATCC strain **700603**) and *E. coli* (ATCC strain **25922**). Luria–Bertani (LB) medium was added to each well in a row on a sterile, Falcon MICROTEST 96-well U-bottom tissue culture plate; 96 μL was added to each well of the first column, and 50 μL was added to all subsequent wells. The compounds or control antibiotic to be tested were added to the first column for a final well volume of 100 μL. The inhibitors were then serially diluted (twofold) across the columns of wells by pipetting and mixing 50 μL of solution. Bacterial cultures were grown to mid-log phase ($OD_{600} = 0.4–0.6$), and diluted to $OD_{600} = 0.004$ using fresh LB medium. 50 μL of the bacterial culture was added to each well of the plate and the plate was then incubated at 37 °C overnight (~14 h) without shaking. For each compound or antibiotic control, the first clear well with no visible signs of growth was reported as the MIC value. Medium from each clear well was then inoculated onto plates of LB agar medium that were incubated overnight. The first clear-medium dilution from which no bacterial colonies could be grown was reported as the MBC value.

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