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Modification of Triclosan Scaffold in Search of Improved Inhibitors for Enoyl-Acyl Carrier Protein (ACP) Reductase in *Toxoplasma gondii*

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Abstract

Through our focused effort to discover new and effective agents against toxoplasmosis, a structure-based drug design approach was utilized to develop a series of potent inhibitors of the enoyl-acyl carrier protein (ACP) reductase (ENR) enzyme in *Toxoplasma gondii* (*Tg*ENR). Modifications to positions 5 and 4' of the well-known ENR inhibitor triclosan afforded a series of 29 new analogs. Among the resulting compounds, many showed high potency and improved physicochemical properties in comparison with the lead. The most potent compounds **16a** and **16c** have IC₅₀ values of 250 nM against *Toxoplasma gondii* tachyzoites without apparent toxicity to the host cells. Their IC₅₀ values against the recombinant *Tg*ENR were 43 and 26 nM, respectively. Additionally, 11 other analogs in this series had IC₅₀ values ranging from 17 to 130 nM in the enzyme-based assay.

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With respect to their excellent *in vitro* activity as well as improved drug-like properties, the lead compounds **16a** and **16c** are deemed to be an excellent starting point for the development of new medicines to effectively treat *Toxoplasma gondii* infections.

Keywords

Enoyl Reductase; Inhibitors; Medicinal Chemistry; *Toxoplasma gondii*; triclosan

Introduction

Toxoplasma gondii (*T. gondii*) is an apicomplexan protozoan responsible for toxoplasmosis, an infectious disease of warm-blooded animals, including humans.^[1] *Toxoplasma gondii* infects about one third of the world's human population and can cause substantial morbidity and mortality.^[2] Infection in humans occurs mainly by consumption of meat infected with tissue cysts or by ingestion of oocysts in food or water contaminated with the feces of cats.^[1] In immunocompetent individuals, acute acquisition of *T. gondii* can be accompanied with fever and adenopathy or other symptoms, but asymptomatic infections can also occur. However, recrudescence in immunocompromised patients can lead to severe pathologic conditions, including lethal encephalitis.^[3] Congenital toxoplasmosis may result in abortion, neonatal death, or fetal abnormalities,^[4] and children congenitally infected with *T. gondii* parasites almost all develop ocular disease during fetal life, in the perinatal period, or at later ages if not treated during fetal life or infancy.^[5] Several distinct stages are involved in *T. gondii* life cycle, which is comprised of two phases: sexual and asexual. The former phase takes place only in the primary hosts, which are domestic and wild cats from the Felidae family, whereas the asexual phase can occur in any warm-blooded animal, which serves as the intermediate host for the parasites.^[6, 7] Tachyzoites and bradyzoites are present in the human stage of the *T. gondii* life cycle. Tachyzoites are the obligate intracellular forms of *T. gondii* and their primary goal is to rapidly expand the parasite population within the host cells during acute infections. In contrast, bradyzoites are the latent forms of *T. gondii*, which slowly multiply and develop cysts inside the host cells in chronic infections. Spiramycin can reduce tachyzoites when it is concentrated in the placenta, where it reaches high concentrations. A combination of sulfadiazine and pyrimethamine are used to treat tachyzoites in acute toxoplasmosis but there are no medicines available to eliminate the latent, encysted bradyzoites.^[8]

T. gondii parasites contain a non-photosynthetic relict plastid, called apicoplast.^[9, 10] Small circular genome and biochemical pathways such as isoprenoid and type II fatty acid synthesis systems were detected within this particular organelle.^[11, 12] The mechanism of the apicoplast-localized type II fatty acid synthesis pathway (FAS II) was initially studied in *Plasmodium falciparum* (*P. falciparum*) and *T. gondii*. As a result, all the FAS II proteins have been identified and characterized in these protozoa. In most prokaryotes, and in *Plasmodia* and *Toxoplasma* protozoan, parasites, the conversion of acetyl coenzyme A (acetyl-CoA) to full-length fatty acid chains is an iterative process mediated by discrete mono-functional enzymes, known as FAS II.^[13, 14] On the contrary, the eukaryotic type I fatty acid synthesis system (FAS I) operates as a single multi-functional enzyme that catalyzes all the steps of the pathway. Also acetyl-CoA carboxylase (ACCase), an enzyme responsible for the synthesis of malonyl-CoA, significantly differs in these two systems. The ACCase of prokaryotes consists of four individual subunits linked to a small acyl carrier protein, whereas the ACCase of eukaryotes is a single large multi-domain protein.^[15] The 'prokaryotic' origin of the biochemical pathways inside apicoplasts has provided a plethora of novel drug targets. Since these are fundamentally different from the corresponding systems operating in the human host cells, several enzymes involved in apicomplexan FAS

II became validated molecular targets for the development of potent anti-protozoan drugs.^[11]

The enoyl-acyl carrier protein (ACP) reductase (ENR or FabI) is one of the key enzymes involved in FAS II that reduces, in a nicotinamide adenine dinucleotide (NADH)-dependent manner, enoyl-ACP to acyl-ACP, which is the final and rate-determining step in the fatty acid chain elongation process.^[16] There are three other isoforms of ENR: FabK, FabL and FabV, which are present in bacteria.^[17–19] The *T. gondii* genome contains a single ENR (*Tg*ENR) that mostly resembles the bacterial FabI isoform and, apart from microgametes, is present in all stages of the pathogen's life cycle.^[20–22] This notion strongly indicates that anti-bacterial drugs, which target FabI, can potentially act against protozoan parasites. Indeed, triclosan (structure shown in Box 1) and other known FAS II inhibitors have been demonstrated to effectively target the apicoplast-associated fatty acid synthesis pathway.^[23–26]

Box 1

Chemical structure of triclosan

Triclosan belongs to the 2-hydroxydiphenyl ether family, and is widely used in household goods such as toothpastes, soaps and plastics due to its activity against a broad spectrum of bacteria. It was initially suspected that this compound acts on bacterial cell membranes, but more recently it was revealed to inhibit bacterial lipid synthesis at the enoyl-ACP reduction step mediated by ENR.^[27, 28] The inhibitory activity of triclosan is based on the formation of a ternary complex with ENR and the oxidized form of the cofactor (NAD⁺). This process is reversible, however, its tight binding and very slow dissociation rate make this inhibitor efficacious.^[29, 30] Although the potency of triclosan is in the low nM range for enzyme activity and low microM for the parasite, this compound is unsuitable for oral administration due to its poor pharmacokinetic properties, especially low solubility. Nonetheless, triclosan has been used as a template for the synthesis of a wide variety of analogs.^[31–34] Although some of the derivatives were equally or even more active than lead,^[35–37] most of them were also accompanied with poor physicochemical properties, which made those compounds undesirable for clinical application. In one of the approaches to improve permeability, a prodrug of triclosan was prepared by coupling the diphenyl ether with an octaarginine through a cleavable ester linker. This oligoarginine-triclosan conjugate was efficiently delivered to bradyzoites in cysts *in vitro* and tachyzoites *in vivo*.^[21]

The fact that triclosan directly inhibits FabI,^[38] and the availability of the crystal structure of *Tg*ENR/NAD⁺/triclosan, makes the diaryl ether template an attractive starting point for the design of new and effective *Tg*ENR inhibitors. The work described herein concerns the design, synthesis and biological evaluation of a wide range of triclosan derivatives as potent inhibitors of ENR enzymatic activity in *T. gondii*, and in some cases *P. falciparum*. Computer-aided ADMET (absorption, distribution, metabolism, excretion, toxicity) predictions were used in the design of compounds with improved drug-like properties. We were particularly interested in improving permeability and water solubility of the new triclosan derivatives. This is exemplified in particular with the ADMET predictions for compounds **9c**, **16c**, and **37c**. The *in vitro* screens against purified *Tg*ENR and *T. gondii* tachyzoites allowed us to select interesting candidates for further biological evaluation.

Overall, this work provides significant insights into the discovery of new and effective inhibitors of *Tg*ENR.

Results and Discussion

Design and synthesis of the A ring triclosan derivatives

Chemistry—The triclosan scaffold was modified at positions 5 and 4' with amide, amine, isoxazole, ketone, and triazole groups according to the schemes presented below.

Vanillin (**1**) was readily converted to its acetal **2** by following a literature procedure (Scheme 1).^[39] Reaction of the resultant phenol **2** with 1,3-dichloro-4-fluorobenzene provided the diphenyl ether **3**,^[40] which was subsequently treated with pyridinium *p*-toluenesulfonate (PPTS) to give benzaldehyde **4**.^[41] Sodium borohydride reduction of **4** provided the corresponding benzyl alcohol **5**,^[42] which was subjected to an Appel reaction to give the benzyl bromide **6**.^[43] Reaction^[44] of the bromide **6** with sodium azide provided the desired precursor **7** for the Cu-catalyzed [3+2] cycloaddition reaction with 1-alkynes to afford triazoles **8a-c**.^[45] Subsequent cleavage of the vinyl silyl bond^[46] and methoxy protecting group^[47] gave the final products **9a-c**.

Nucleophilic aromatic substitution of 3-chloro-4-fluorobenzaldehyde with 4-chloro-2-methoxyphenol (**10**) gave aldehyde **11**.^[48] (Scheme 1), which was subsequently converted to the intermediates **15a-c** by following the same protocols as described above. The corresponding 4'-triazole analogs of triclosan, **16a-c**, were obtained by the standard methyl aryl ether cleavage procedure using BBr₃.^[49]

Triclosan derivatives bearing isoxazole groups at positions 5 and 4' were also synthesized (Scheme 2). Intermediates **19a-c** and **23a,b** were prepared by following the Sharpless reference cited above.^[45] Aldehydes **4** and **11** were converted in high yields into the oximes **17** and **21**, respectively. Reaction of these oximes with *N*-chlorosuccinimide (NCS) gave the corresponding imidoyl chlorides **18** and **22**, which were immediately reacted with the appropriate 1-alkynes to provide isoxazoles **19a-c** and **23a,b**, respectively. Tetrabutylammonium iodide/BCl₃ promoted cleavage of the resulting methyl aryl ethers afforded the desired final products **20a-c** and **24a,b**, respectively.

The versatile intermediate **26** was obtained by condensing **25** with 2,4-dichlorophenol (Scheme 3).^[40] Subsequent BBr₃ mediated deprotection provided the 5-cyano derivative **27**. Hydrolysis of **26** under basic conditions^[37] gave the corresponding benzoic acid **28**, which was elaborated further to the amides **29a-c** by following a published procedure.^[50] Deprotection of the methoxy group afforded the 5-amide analogs of triclosan, **30a-d**. Reduction of the nitrile **26** with LiAlH₄ afforded the benzylamine derivative **31**.^[51] Elaboration of **31** via amide bond formation with 5-methyl-3-isoxazolecarboxylic acid followed by demethylation afforded the final product **33**.

The corresponding 4'-modified triclosan analogs **37a-d** were prepared in the same manner as described for the synthesis of compound **33** (Scheme 3). Nucleophilic substitution of the bromide **6** with primary amines and subsequent deprotection provided the 5-benzylamine analogs **39a-c**. Reductive amination^[52] of benzaldehyde **11** with aniline and hexylamine, respectively, gave the secondary amines **40a,b**, which after standard deprotection reactions allowed the isolation of the 4'-benzylamine analogs **41a,b**. Addition of ethylmagnesium bromide and phenylmagnesium bromide to the nitrile **26** followed by acidic quenching provided the corresponding ketones **42a** and **42b**, respectively.^[53] Subsequent cleavage of the methoxy protecting group afforded the final compounds **43a** and **43b**.

The newly synthesized triclosan analogs were evaluated for their inhibitory activity (MIC₅₀) against *T. gondii* tachyzoites (measured as diminished uptake of [³H] uracil or fluorescence with YFP transfected parasites [please see methods], Table 1).

The toxicity for the Human Foreskin Fibroblasts (HFF), in which the tachyzoites were cultured and compounds were tested, was determined using the highest concentration of the tested compound (10 μ M) on host cells using 10% DMSO (v/v) as a control, and assessing replication of nonconfluent HFF in a [³H] thymidine assay.

Additionally, the compounds were screened in duplicate at a concentration of 1 μ M for inhibition of *Tg*ENR enzymatic activity. Analogs with significant inhibition (typically >90 % at 1 μ M) were subsequently assayed in triplicate at 11 concentrations to determine their IC₅₀ values. The *in vitro* parasite, human host cell, and enzyme data along with predictions for the selected ADMET parameters are presented in Table 1.

The mode of action of triclosan is well understood and is conserved throughout the ENR family. The inhibitor acts by slow and tight binding to the ENR/NAD⁺ complex, which makes it very effective. The π -stacking interactions between the phenol ring (A) and the oxidized nicotinamide, as well as the hydrogen bonding between the triclosan hydroxyl group, the ether linkage, the conserved tyrosine residue and the 2' hydroxyl of NAD⁺, were determined to be crucial for the efficacy of triclosan.⁵⁴ Bearing this in mind, we addressed our interest to modify the *para* positions of both aromatic rings of triclosan while the ring A hydroxyl group and the ether linker were kept intact. The rationale for modification at the 5 position of phenolic ring A comes from the fact that apicomplexan ENR contains a fully conserved alanine residue in close proximity to the *para* position of ring A. For comparison, its plant and bacterial homologues contain bulky methionine, leucine or isoleucine residues in that region. This change in amino acids results in reduction of the van der Waals forces and creates more space in the enzyme's binding pocket which, in turn, provides the opportunity to introduce various substituents at the 5 position in search of ENR inhibitors with improved activity.^[54] On the other hand, the 4' position on the dichlorobenzene ring (B) points out towards the inhibitor's entry portal, therefore modification of this position with bulky substituents might allow for an increase in enzyme-inhibitor interactions while improving inhibitor properties such as solubility, work that has been described.^[54a]

While modifying the triclosan scaffold with various groups, we also paid attention to the drug-like properties of these new analogs. During the design process of new inhibitors, we took into account the poor aqueous solubility of the lead compound and the fact that the target enzyme is located inside the four-membrane apicoplast. Therefore, before we started the synthesis of analogs, ADMET prediction software (from ACD/Labs) was used to select compounds with improved permeability, water solubility and other important physicochemical parameters, such as ClogP.

We were pleased to find that compounds bearing 4-butyltriazole (**16a**) and unsubstituted triazole (**16c**) groups at the 4' position of the triclosan scaffold were the most active analogs in this series against *T. gondii* tachyzoites. On average, **16a** and **16c** were four-fold more active than triclosan (Table 1). Both compounds also showed high inhibition of *Tg*ENR enzymatic activity (90 and 91%, respectively, at 1 μ M compound concentration) with IC₅₀ values comparable to that of triclosan (43 and 26 nM, respectively, vs 17 nM for triclosan). The ADMET suite predicted compound **16c** to possess improved ClogP, water solubility (Sw) and Caco-2 permeability values in comparison with triclosan, which in turn would suggest increased access of the compound to the biological target. On the other hand, compound **16a** has good potency but its predicted ADMET properties are less favorable in comparison to **16c**. The improved drug-like properties along with high *in vitro* activity make

analog **16c** an attractive lead candidate for further optimization. Compounds possessing 4-phenyltriazole (**16b**), 5-methylisoxazole (**37a**), and hexylamine (**41a**) groups at the 4' position as well as the analogs with attached cyclohexylamine (**39b**) and hexylamine (**39c**) substituents at the 5 position displayed activity comparable to or better than that of triclosan ($\text{MIC}_{50} = 0.25 \mu\text{M}$, Table 1) in the parasite-based assay. Compounds **16b** and **37a** moderately inhibited the enzyme to the extent of 82 and 88%, respectively, at a concentration of $1 \mu\text{M}$. However, the remaining amine analogs bearing *N*-cyclohexyl or *N*-hexyl substituents (**39b**, **c** and **41a**) were completely inactive in the enzyme-based assay. These results suggest that the amine analogs may owe their activity in the parasite assay to interaction with targets other than the *Tg*ENR.

The growth of tachyzoites was only weakly inhibited ($\text{MIC}_{50} = 10 \mu\text{M}$) by compounds bearing the corresponding unsubstituted triazole (**9c**), 5-methylisoxazole (**33**) and aniline (**39a**) groups at position 5. Additionally, derivatives **24a**, **24b** and **33** had robust enzyme activity. Derivatives **24a** and **24b** had IC_{50} values of 18 and 28 nM, respectively. The 5-methylisoxazole derivative **33** inhibited the enzyme to the extent of 96% (at a concentration of $1 \mu\text{M}$) and its IC_{50} was 19 nM, which was one of the lowest values in this series of analogs. In addition, the favorable predictions of compound **33**'s physicochemical properties such as ClogP, water solubility, and Caco-2 permeability make this molecule a viable lead for further rounds of medicinal chemistry optimization. Analogs **9c** and **39a** showed good inhibition of *Tg*ENR as well, both with IC_{50} values of 130 nM.

Compounds containing modifications at the 5 position of the triclosan core returned surprising results. Derivatives bearing 4-butyltriazole (**9a**) and 4-phenyltriazole (**9b**), but not cyano (**27**) groups were inactive in the parasite assay, however, all of them efficiently inhibited *T. gondii* ENR (93 – 97% at $1 \mu\text{M}$ compound concentration) and their IC_{50} values were in the range of 24 – 54 nM (Table 1). The same trend was observed for the analogs with 5-phenylisoxazole (**37b**), (3-methyl-isoxazo-5-yl)-acetamide (**37c**), and aniline (**41b**) attached at the 4' position. The lack of activity against parasites of these compounds might be attributed to their low solubility (supported by the calculations), and/or restricted access to the target enzyme located in the apicoplast. The predicted poor permeability for some of these compounds through the parasite cell membrane might be responsible for their lack of ability to inhibit the growth of *T. gondii* parasites. This underlines the fine balance between increasing the inhibitors solubility whilst not affecting its ability to cross the parasite cell membrane. Reasonable activity against purified *Tg*ENR was observed for an analog bearing the 6-hydroxynaphthalene group at the 4' position (**37d**). Nonetheless, its high ClogP value and poor water solubility prevent this compound from being subjected to further optimization. Ketones **43a** and **43b** showed only weak activity in the enzyme based assay while being ineffective to inhibit parasite whole cell growth, thus these analogs are considered to be poor *Tg*ENR inhibitors. The triclosan analogs bearing 5-butylisoxazole (**20a**) and 5-phenylisoxazole (**20b**) were completely inactive in both assays. However, compound **20c** showed limited activity only in the parasite assay ($\text{MIC}_{50} = 7 \mu\text{M}$, Table 1). Disappointingly, all the amide-modified analogs **30a-d** did not show any activity in either of these assays. The same result was found for compound **8d**, however, in this case the lack of activity can be easily explained by the presence of the methoxy instead of hydroxyl group on ring A.

To better understand the activity of the above series of compounds, we used molecular docking to reveal possible interactions and conformational changes imposed by the novel triclosan analogs in the binding pocket of *Tg*ENR. It was observed that compound **16c** binds to *Tg*ENR in a mode similar to triclosan (Figure 1A), therefore the crucial interactions of the diaryl ether core with cofactor and enzyme are preserved. However, the alignment of **16c** in

the protein's active site is slightly disrupted due to the presence of the bulky triazole group at the 4' position (Figure 1B).

The phenol ring A of **16c** is sandwich-positioned towards the NAD⁺ cofactor, which allows the two components to interact via π -stacking in the same manner seen with triclosan. The phenolic hydroxyl group on ring A is in close proximity to the conserved Tyr189 residue and the 2' hydroxyl group of NAD⁺, thus they interact through hydrogen-bonding. The ether linkage is beyond the H-bond distance from the 2' hydroxyl group of NAD⁺ and therefore is not engaged in any interactions. The aromatic ring B of **16c** is slightly reoriented in comparison with the actual position of ring B for triclosan when bound in the active site (Figure 1B). Importantly, however, is that two new hydrogen-bonding interactions are created between the nitrogen atoms at the 2 and 3 positions of the triazole ring with the amide NH of Asn130 and Gly131. These new H-bonding interactions of compound **16c** with surrounding *Tg*ENR residues may be decisive for its high inhibitory activity in the enzyme-based assay. Alternatively, the molecular modeling revealed that the triazole group might rotate and take a position that is favorable for H-bonding interaction with Ala231. Additionally, desirable *in silico* ADMET properties may account for the high activity of this analog in the parasite-based assay.

The two 4'-triazole modified compounds **16a** and **16b** bind in a very similar mode as analog **16c** with higher IC₅₀ values. This is attributed to the presence of substituents at the 4 position of the triazole ring. The 4-butyltriazole **16a** had IC₅₀ values 1.6-fold higher than that of **16c** (43 vs 26 nM). The *n*-butyl chain of **16a** introduces steric hindrance, which affects the alignment of ring A in the active site. The aromatic ring A is slightly shifted away from the NAD⁺ unit, therefore, the π -stacking interactions are weakened, and hence the binding energy for this inhibitor is increased. For the third triazole derivative in this series, (**16b**), its bulky 4-phenyltriazole group imposed dramatic effects on the binding mode, and thus the *Tg*ENR enzyme was inhibited only to the extent of 82% at a concentration of 1 μ M. The rigid phenyl group may significantly affect the position of the aromatic ring B of **16b** such that it no longer makes the optimum packing interactions seen with triclosan. Additionally, the H-bonds between the triazole group, Asn130 and Gly131 are missing, which accounts for the decreased activity of this inhibitor.

Derivative **33** bearing the 5-methylisoxazole group at the 5 position displayed excellent activity against recombinant *Tg*ENR. This compound inhibited the enzyme by 96% at a concentration of 1 μ M and its IC₅₀ was found to be 19 nM. Molecular docking studies using FlexX (version 2.0.2 Linux64 provided by BioSolveIT GmbH) and the X-ray crystal structure of *T. gondii* ENR in complex with triclosan suggested that, compound **33** is accommodated in the substrate-binding site in an unusual manner. In this case, the isoxazole ring partly occupies the same space as does ring A of triclosan and therefore, is able to interact via π -stacking with the NAD⁺ cofactor. Moreover, the amide oxygen atom of **33** is hydrogen bonded to the 2' hydroxyl group of NAD⁺, which provides additional stabilization for this moiety in the active site of the protein. The isoxazole methyl substituent points out in the same direction as the Cl atom on ring A of triclosan and therefore, might efficiently fill out the hydrophobic cavity in the substrate-binding site. The phenolic ring A of **33** is significantly shifted and is situated almost perpendicular in comparison to the original position of ring B of triclosan when bound to the enzyme. Nonetheless, its hydroxyl group contributes to the hydrogen-bonding interactions with the carbonyl oxygen of Ala129. The 2',4'-dichlorobenzene ring (B) is located outside the binding pocket and does not seem to be involved in any interactions. Such accommodation of compound **33** facilitates its efficient packing into the active site of *T. gondii* ENR, which in turn corresponds to the high inhibitory activity of this analog. This apparent reverse binding mode was also seen for **37b**, which in possessing an aromatic substituent on the isoxazole ring also has a significantly

bulky extension on the A-ring. Since the only compounds which display this apparent reverse binding mode contain large extensions on the A-ring, it is likely that the modeling is not taking into account the flexibility within the binding site.^[33] By modeling the inhibitor into a crystal structure whose binding site has been extended through binding a bulkier inhibitor this class of inhibitor was shown to bind with a similar mode as triclosan. Although we cannot, without a co-crystal structure rule out the ability of compounds **33** and **37b** to bind in a reverse mode, as predicted by the FlexX program it would seem likely that the natural plasticity of the ENR binding site can accommodate the bulkier nature of the A ring in **33** and **37b** negating the need for a reverse binding mode. Compounds **37a** & **37c** have a smaller methyl substituent on the isoxazole ring compared to **37b**, and are predicted to bind to the enzyme in the same fashion as triclosan (Figure 1C). Their aromatic ring A is involved in the π -stacking and H-bonding interactions in the active site while the isoxazole ring is positioned towards the inhibitor's entrance. Additional stabilization arises from the H-bonds between the compound's amide oxygen and the amide NH of Asn130 and Gly131.

After identifying the molecular interactions defining the activity of the most potent compounds in this series (**16a**, **16c** and **33**), we were interested in explaining the activity or its lack thereof displayed by the other analogs. Triazole analogs (**9a-c**) interact with the enzyme through a different binding mode compared to triclosan. In this particular case, the activity of compounds **9a-c** is determined by the triazole group, which binds at the inhibitor's entry portal to NH groups of Asn130 and Gly131 via hydrogen-bonds. As a result, the most internal part of the binding pocket is occupied by the 2,4-dichlorobenzene ring B whereas ring A is shifted away towards the entry portal and occupies the space between ring B and the triazole group. Nonetheless, the presence of *n*-butyl and phenyl substituents at the 4 position of the triazole ring (**9a**, **9b**) imposes significant positional change on ring A in the binding site. The phenolic ring A of compounds **9a** and **9b** is positioned perpendicular (when compared to ring B of triclosan) with its hydroxyl group being directed toward Tyr189 and the nicotinamide ribose of NAD⁺, and thus is able to interact with those units via H-bonding. Although the unsubstituted triazole group of analog **9c** also interacts through H-bonding with Asn130 and Gly131, it does not impose the favorable position of ring A in the binding pocket and therefore its phenolic group is not involved in any H-bonding interactions with the proximal residues. As a result, the IC₅₀ values of compounds **9a** and **9b** are approximately 3 and 2-fold lower, respectively, than that of analog **9c** (i.e. 38 and 54 nM vs 130 nM, Table 1).

The ketone compounds **43a** and **43b** showed only weak inhibition of the enzyme. According to the modeling results, these compounds bind in reverse mode to that of triclosan, and the visible H-bond interactions between the ketone oxygen, Asn130, and Gly131 do not seem to improve the inhibitory activity of these compounds. The amine analogs **39a** and **41b** provided mixed results, with the latter being the more active of the two compounds versus the purified enzyme. Compound **39a** also binds in a reverse mode compared to that of triclosan. The NH hinge is engaged into H-bonding with the carbonyl oxygen of Ala129 which affects the biarylether moiety alignment in the active site, though π -stacking between ring B and NAD⁺ as well as H-bonding between the ring A hydroxyl group and Tyr189 are observed. On the other hand, the biaryl-ether moiety of **41b** is well packed in the substrate binding site (nearly the same as triclosan), which in turn seems to be responsible for the high activity (IC₅₀ = 31 nM) of this derivative. Although the isoxazole compounds **20a-c** as well as the amide analogs **30a-d** were predicted to dock reasonably well, none of these compounds turned out to be highly active in either biologic assay. We postulate that the lack of a flexible linker between the isoxazole group and aromatic ring A in compounds **20a-c** or the presence of a rigid and polar amide bond in the case of compounds **30a-d** introduces a steric clash and therefore prevents these inhibitors from optimal accommodation into the enzyme's binding pocket. This hypothesis can be partly supported by analogs **24a** and **24b**,

whose isoxazole rings are rigidly appended at the 4' position of the scaffold but their inhibitory activity is not lost. It is believed that the 4'-isoxazole group allows the biaryl-ether moiety of **24a** and **24b** to be accommodated in the enzyme's active site.

Encouraged with these promising *in vitro* results, we selected compounds **16c** and **37c** for further biological evaluation in the *T. gondii* mouse infection model (Figures 2–4). It turned out that neither of those analogs reduced the parasite burden at a dose at which triclosan was effective (10 mg/kg) (Figures 2 and 3). However, at a higher dose (75 mg/kg) both compounds protected the mice by decreasing parasite burden (Figure 4). These compounds were 10-fold less toxic than triclosan in mice. To further investigate the cause of the decreased *in vivo* potency of **16c**, experimental ADMET testing of this compound was performed by a Contract Research Organization (CRO). The following experiments were performed: Caco-2 permeability, intrinsic clearance in human liver microsomes, and intrinsic clearance in human cryopreserved hepatocytes. Briefly, the Caco-2 permeability of **16c** was 26.3×10^{-6} cm/s. For comparison, the reference compounds colchicines, labetalol, propranolol, and ranitidine had the following values for Caco-2 permeability: 0.1; 8.5; 51.1; and 0.4×10^{-6} cm/s, respectively.

The half-life of this compound in cryopreserved hepatocytes was 29 min. After only 60 min, only 20% of the compound was left unmetabolized, whereas after 120 min only 5% of the compound remained intact. However, the half-life of **16c** in liver microsomes was over 60 min.

Although Caco-2 permeability of analog **16c** is not as high as predicted by the *in silico* modeling (Table 1), the above data indicate that in general this molecule has good permeability. However, due to extensive hepatic metabolism the efficacy of **16c** (and related analogs) is significantly reduced. This would explain the need for higher doses (i.e. 75 mg/kg) in order to observe the protective effect of this compound in infected mice (Figure 4).

Additionally, some of the triclosan analogs, which were initially tested against *T. gondii*, were re-purposed for testing against *P. falciparum* strain D6 (CDC/Sierra Leone) and TM91C235 (WRAIR, Thailand, chloroquine resistant). The data are presented in Table 2.

It has been previously shown that the FASII pathway is non essential for malaria during the erythrocytic stage [75,76]. In fact, these studies showed that *PfENR* enzyme was successfully knocked out and the parasites were viable in *in vitro* culture, showing that this enzyme is dispensable. The activity results (Table 2) clearly identify *P. falciparum* strain D6 to be more sensitive to these analogs than strain TM91C235. The triazole analog **9c** showed very modest activity against strain D6 of *P. falciparum*. Among the amide-modified compounds **30a-d**, only the analog bearing the *N*, *N*-dibutyl substituent (**30b**) showed promising inhibitory activity. Those results indicate that aromatic (**30a**), rigid heterocycloaliphatic (**30c**), or even slightly longer aliphatic, e.g. *n*-hexyl (**30d**) substituents at position 5 seem to be detrimental for parasite inhibition. It is also worth noting that compound **30b** has the highest ClogP value among the tested **30a-d** analogs, which may imply that lipophilic molecules easier reach the molecular target and therefore display better inhibitory activity. Similar levels of modest inhibitory activity were displayed by compound **9c** and by the aniline analog **39a**. Surprisingly, the derivative bearing the (cyclohexylamino)methyl substituent at the 5 position of the triclosan scaffold (**39b**) showed excellent inhibition against this strain with an IC₅₀ value of 0.03 μ M (Table 2). The high activity of compound **39b** against *P. falciparum* parasites makes this analog one of the most potent triclosan derivatives possessing antimalarial activity reported to date. The other amine analogs **39a** and **39c** showed relatively weak antimalarial activity. The comparison of **39a** and **39b** is particularly illuminating since these compounds display a difference of over 2 orders of

magnitude in antimalarial activity, yet only differ in the degree of saturation of the six-carbon ring at the 5' position.

In case of TM91C235 strain of *P. falciparum* only two compounds showed the desired inhibitory activity, the amide derivative **30b** and the amine analog **39b**. Although those analogs were less potent against this strain than against the D6 strain, the fact that they are potent against both strains of *P. falciparum* strongly suggest that both derivatives are likely to be affecting the same molecular target. Furthermore, given the poor *Tg*ENR inhibition by these compounds (44% and 34% at 1 μ M, respectively) it is likely that ENR inhibition is not responsible for antiparasitic activity. Regardless of their mode of action, compounds **30b** and **39b** seem to be an attractive starting point for further development of agents to successfully treat malaria.

Conclusions

We successfully carried out the rational design and synthesis of new *Tg*ENR inhibitors based on the triclosan scaffold. Computational tools were used to design *Tg*ENR inhibitors with improved drug-like properties. Of the 29 compounds synthesized, 2 had antiparasitic MIC₅₀ values of 250 nM, which is approximately ten-fold better than that of the lead compound, triclosan. Additionally, 14 analogs in this series had IC₅₀ values ranging from 17 to 130 nM against the recombinant *Tg*ENR enzyme. Molecular docking revealed that the synthesized compounds can bind to the target protein in a similar or possibly a reversed mode compared to that of triclosan, depending on the nature of the substituents appended at 5 and 4' positions of the triclosan scaffold. The most promising analogs (**16c** and **37c**) were tested for their *in vivo* efficacy in the *T. gondii* mouse infection model, with limited efficacy. Also a small subset of these compounds were evaluated against *P. falciparum* strain D6 and TM91C235. One analog (**39b**), among 10 tested, showed excellent activity against the D6 strain, which makes it a promising lead candidate for further research. Overall, this work provides new insights into development of new inhibitors targeting *Tg*ENR and an off target in *P. falciparum*. The approach presented herein utilizes the increase in space observed within the active site of parasitic ENR compared with the homologous bacterial enzyme. As illustrated by our results, this strategy leads to highly potent inhibitors against ENR enzyme in *T. gondii*.

Experimental Section

Molecular docking and ADMET calculations

The ADMET calculations were performed by using PhysChem, ADME, and Tox Suite (version 2012) provided by Advanced Chemistry Development, Inc. All calculation parameters were performed at default values of the predictor.

Biology

Inhibition of *Tg*ENR activity *in vitro*—Recombinant *Tg*ENR was purified as described previously.^[57] An assay of *Tg*ENR enzymatic activity used previously^[37] was adapted for use in 96-well plates. The activity of *Tg*ENR was monitored by consumption of NADH ($\epsilon_{340} = 6220 \text{ M}^{-1}\text{cm}^{-1}$) with a SpectraMax M2 plate reader. Reactions were carried out with a final volume of 100 μ L in 96 well Corning UV plates. Initially, 10 μ L of 1 mM Crotonyl-CoA (Sigma) was placed in each well with 1 μ L of DMSO (or compound dissolved in DMSO). Then a reaction mixture containing a final concentration of 5nM *Tg*ENR, 100 mM Na/K Phosphate pH 7.5, 150 mM NaCl, and 100 μ M NADH was added row by row with a 12-channel pipetter to initiate the assay. Following a brief mixing period, the absorbance in each well was monitored at 35-second intervals for 15 minutes. The first

column of each plate contained blank reactions with 1% DMSO which served as baseline activities for each row of the assay plate. The last column of each plate contained the potent inhibitor triclosan in concentrations ranging from 10 μ M to 610 pM (serial dilutions by factors of 4), which served as a positive control for *Tg*ENR inhibition. Enzymatic activity was determined by comparing the slopes of the absorbance curves for each well to those of the blanks in the first column of the plate. Compounds were initially screened in duplicate at a concentration of 1 μ M. Compounds with significant inhibition (typically >90% at 1 μ M) were further analyzed to determine IC₅₀ values. These values were determined in triplicate with each replicate consisting of 11 inhibitor concentrations ranging from 10 μ M to 170 pM (serial dilutions by factors of 3). Nonlinear regression analysis was performed using GraphPad Prism software. The Z-factor for this assay⁵⁸ was calculated from data spanning 25 plates and was found to be 0.65.

Molecular Docking

Molecular docking studies were performed using FlexX (version 2.0.2 Linux64 provided by BioSolveIT GmbH). The X-ray crystal structure of *T. gondii* ENR in complex with triclosan was taken from Brookhaven Protein Data Bank, PDB code: 2O2S. The synthesized molecules were modeled in the active site of *Tg*ENR, which was assigned as a radius of 8 Å. Other docking parameters were left as default. The obtained docking scores were analyzed and pictured by employing Benchware 3D software. Additional docking was analyzed using the bound triclosan as a guide within the *T. gondii* structure.

Inhibition of *Toxoplasma gondii* tachyzoites *in vitro* and toxicity assays—Type I RH tachyzoites expressing Yellow Fluorescent Protein (YFP) were kindly provided by Dr. Boris Streipen (University of Georgia) and cultured in monolayers of Human Foreskin Fibroblast (HFF) cells. Parasites were separated from HFF cells by passage through a 25-gauge needle twice. Confluent HFF cells in 96-well plates (Falcon 96 Optilux Flat-bottom) were inoculated with 3,500 parasites per well. Parasites were allowed to infect cells for one hour before inhibitory compounds and control media were added. After 72 hours, parasite proliferation was assessed using a [³H] uracil incorporation assay,^[59] or a YFP fluorescence assay, where relative fluorescence of parasite samples was measured using a Synergy H4 Hybrid Reader (BioTek) and Gen5 1.10 software. All compounds and control solutions were tested in triplicate exemplars. Biological replicates of each experiment were performed at least twice, as described previously.^[21, 37, 59–72] Effect of compounds on replication of *T. gondii* within infected fibroblasts was determined using assays described previously.^[21, 23, 37, 59–72] The primary goal of these studies was to identify compounds that were inferior to or superior to triclosan in an attempt to identify compounds that had improved efficacy both in the parasite and enzyme assays and their predicted ADMET properties so that the most promising compounds could be progressed. Synthesized compounds were initially tested at 10, 1, 0.1 and 0.01 μ M concentrations. Those compounds that inhibited replication by 50% between 1 and 10 μ M were tested at 1.25, 2.5, 5, and 10 μ M. Those compounds that inhibited replication by 50% between 0.1 and 1 μ M were tested at 0.1, 0.25, 0.5, and 0.75 μ M. In each assay these results were compared with those for DMSO control and triclosan. Other internal controls included a curve obtained with varying concentrations of parasites to confirm that each assay detected differing numbers of parasites, and cultures treated with a known inhibitory concentration of pyrimethamine and sulfadiazine as a positive control. This was to demonstrate that in each assay we could detect inhibition of parasites with a known standard of care inhibitor, as in earlier work.^[21, 23, 37, 59–72] Each experimental determination for each concentration of compound or control was performed in triplicate and a mean and standard deviation calculated. A curve displaying the results was made allowing comparison of the data between experiments visually, directly. Independent, separate, biological replicate experiments were performed at

least two separate times for each compound to confirm results were reproducible and consistent. Inhibitory index was calculated as = [RFU with this [compound] – RFU control fibroblasts/RFU DMSO control – RFU control fibroblasts] × 100. MIC₅₀ was the concentration that inhibited replication by 50%. This was using a log scale curve based on the empirical data for compounds with values between 0.1 and 10.0 μM tested at the concentrations specified above. Data also are presented for toxicity assays assessing viability of HFF host cells for this obligate intracellular parasite. These assays are performed in parallel with inhibition of replication studies. These data in the column entitled “HFF toxicity” allow proper interpretation of concomitant efficacy data because if there were toxicity to HFF at the concentration at which a compound appeared to be effective the data in the assay could not be interpreted.

Toxicity for host cells was evaluated by [³H] thymidine incorporation assays using non-confluent HFF cell, as well as by a WST-1 cell viability assay, a commercially available calorimetric kit (Roche). Confluent HFF cells were treated under the same conditions as challenge assay, without parasite infection. After 72 hours, absorbance at 420nm was measured using a Synergy H4 Hybrid Reader (BioTek) and Gen5 1.10 software.

Inhibition of *Plasmodium falciparum* parasites *in vitro*—The Malaria SYBR Green I-Based Fluorescence (MSF) Assay is a microtiter plate drug sensitivity assay that uses the presence of malarial DNA as a measure of parasitic proliferation in the presence of anti-malarial drugs or experimental compounds. As the intercalation of SYBR Green I dye and its resulting fluorescence is relative to parasite growth, a test compound that inhibits the growth of the parasite will result in a lower fluorescence. D6 (CDC/Sierra Leone), TM91C235 (WRAIR, Thailand), and W2 (CDC/Indochina III) laboratory strains of *P. falciparum* were used for each drug sensitivity assessment. The parasite strains were maintained continuously in long-term cultures as previously described by Johnson *et al.*^[73] Pre-dosed microtiter drug plates for use in the MSF assay were produced using sterile 384-well black optical bottom tissue culture plates containing quadruplicate 12 two-fold serial dilutions of each test compound or mefloquine hydrochloride (Sigma-Aldrich Co., Catalog #M2319) suspended in DMSO. The final concentration range tested was 0.5 – 10000 ng/ml for all assays. Predosed plates were stored at 4°C until used, not to exceed five days. No difference was seen in drug sensitivity determinations between stored or fresh drug assay plates (data not shown). A batch control plate using chloroquine (Sigma-Aldrich Co., Catalog #C6628) at a final concentration of 2000 ng/ml was used to validate each assay run. The Tecan Freedom Evo liquid handling system (Tecan US, Inc., Durham, NC) was used to produce all drug assay plates. Based on modifications of previously described methods,^[73, 74] *P. falciparum* strains in late-ring or early-trophozoite stages were cultured in the predosed 384-well microtiter drug assay plates in 38 μl culture volume per well at a starting parasitemia of 0.3% and a hematocrit of 2%. The cultures were then incubated at 37°C within a humidified atmosphere of 5% CO₂, 5% O₂ and 90% N₂, for 72 hours. Lysis buffer (38 μl per well), consisting of 20mM Tris HCl, 5mM EDTA, 1.6% Triton X, 0.016% saponin, and SYBR green I dye at a 20x concentration (Invitrogen, Catalog #S-7567) was then added to the assay plates for a final SYBR Green concentration of 10x. The Tecan Freedom Evo liquid handling system was used to dispense malaria cell culture and lysis buffer. The plates were then incubated in the dark at room temperature for 24 hours and examined for the relative fluorescence units (RFU) per well using the Tecan Genios Plus (Tecan US, Inc., Durham, NC). Each drug concentration was transformed into Log[X] and plotted against the RFU values. The 50% and 90% inhibitory concentrations (IC₅₀s and IC₉₀s, respectively) were then generated with GraphPad Prism (GraphPad Software Inc., San Diego, CA) using the nonlinear regression (sigmoidal dose-response/variable slope) equation.

***In vivo* experiments**

These were performed as described previously.^[70] Permission was obtained from the relevant national or local authorities. The institutional committees (IACUC and British Home Office) that approved the experiments have the following assurance number, A3523-01.

Statistics

These were performed as described above and previously.^[71] For *in vivo* experiments, an initial ANOVA was performed when $P < 0.000001$, pairwise *T* comparisons were compared using Student *t* test.

General Information for Chemistry

All chemicals and solvents were purchased from Sigma-Aldrich and/or Fisher Scientific, and were used without further purification. Anhydrous THF and CH_2Cl_2 were obtained by distillation over sodium wire or CaH_2 , respectively. All non-aqueous reactions were carried out under an argon atmosphere with exclusion of moisture from reagents, and all reaction vessels were oven-dried. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer at 400 MHz and 100 MHz, respectively, and were referenced to the residual peaks of CHCl_3 at 7.26 ppm or $\text{DMSO}-d_6$ at 2.50 ppm (^1H NMR), and CDCl_3 at 77.23 ppm or $\text{DMSO}-d_6$ at 39.51 ppm (^{13}C NMR). Chemical shifts were reported in parts per million downfield of TMS and the following abbreviations used to denote coupling patterns: s = singlet; d = doublet, t = triplet, q = quartet, br = broad, fs = fine splitting). Mass spectra were measured in the ESI mode at an ionization potential of 70 eV with an LC-MS MSD (Hewlett Packard). HRMS experiments were performed on a Shimadzu LCMS-IT-TOF spectrometer. TLC was performed on Merck 60 F₂₅₄ silica gel plates. Column chromatography was performed using Merck silica gel (40–60 mesh). Preparative HPLC was carried out on an ACE 5 AQ column (150 × 20 mm), with detection at 254 and 280 nm on a Shimadzu SPD-10A VP detector; Method I: flow rate = 17 ml/min; gradient from 25% to 100% methanol in water within 40 min; Method II: flow rate = 17 ml/min; gradient from 8% to 100% methanol in water within 40 min. Purities of all final compounds were established by analytical HPLC, which was carried out on an Agilent 1100 HPLC system with a Synergi 4 μ Hydro-RP 80A column, with detection at 254 nm on a variable wavelength detector G1314A; Method I: flow rate = 1.4 ml/min; gradient from 30% to 100% methanol in water within 16 min (both solvents containing 0.05 vol % of CF_3COOH); Method II: flow rate = 1.4 ml/min; gradient from 10% to 100% methanol in water within 21 min (both solvents containing 0.05 vol % of CF_3COOH).

General Procedures

General Procedure A: Preparation of 1,3–Disubstituted Triazoles

The compounds were prepared by following a literature procedure.⁴⁵ The appropriate benzyl azide (0.50 mmol, 1.0 equiv), sodium ascorbate (0.05 ml of a 1.0 M freshly prepared solution in deionized water, 0.050 mmol, 0.1 equiv), CuSO_4 (1.25 mg freshly dissolved in 50 μL of deionized water, 0.050 mmol, 0.1 equiv), and 1-alkyne (0.50 mmol, 1.0 equiv) were suspended in *t*-BuOH/ H_2O (1:1, 2.0 ml). The reaction mixture was stirred at room temperature for 14 – 21 h. The crude reaction mixture was poured onto water (25 ml) and extracted with EtOAc (2 × 25 ml). The combined organic phases were washed with saturated aqueous solution of NH_4Cl (30 ml), 10% aqueous solution of sodium tartrate (30 ml), and water (30 ml). The organic phase was dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to give crude material of sufficient purity to be used in the next step without additional purification.

General Procedure B: Cleavage of Methyl Ethers with *n*-Bu₄NI – BCl₃

A literature procedure was followed.⁴⁷ In a 25 ml round-bottom flask were placed the starting material (0.50 mmol, 1.0 equiv), *n*-Bu₄NI (462 mg, 1.25 mmol, 2.50 equiv), and dry CH₂Cl₂ (4 ml). After stirring for 5 min at room temperature, the reaction mixture was cooled to –78 °C, and a solution of BCl₃ (1.25 ml of a 1.0 M solution in hexane, 1.25 mmol, 2.50 equiv) was added dropwise. Stirring was continued at the same temperature for 15 min before removing the cooling bath. The reaction mixture was stirred at room temperature for 2.5 – 3 h before quenching by addition of a mixture of ice and water (around 5 ml), and stirring was continued for further 30 min. The mixture was poured into saturated aqueous solution of NaHCO₃ (25 ml), and the products were extracted into CH₂Cl₂ (2 × 25 ml). The combined organic phases were washed with water (25 ml), dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give crude material as an oil or solid, which was subsequently purified by preparative HPLC.

General Procedure C: Cleavage of Methyl Ethers with BBr₃

A literature procedure was followed.^[49] The starting material was placed into a round-bottom flask, which was evacuated and refilled with Ar, followed by addition of dry CH₂Cl₂ (to prepare a 0.1 M solution). The flask was cooled to –78 °C, and BBr₃ (1.0 M solution in CH₂Cl₂, 5 equiv) was added dropwise. The reaction mixture was warmed gradually to room temperature within 1 h, and stirring was continued for further 2.5 – 3 h. The reaction was terminated by addition of water, and the products were extracted into CH₂Cl₂ (2 × 25 ml). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give crude material, which was subsequently purified by preparative HPLC.

General Procedure D: Preparation of 3,5-Disubstituted Isoxazoles

The compounds were prepared following a literature procedure.^[45] To the stirred solution of a carboximidoyl chloride (792 mg, 2.0 mmol) in *t*-BuOH/H₂O (1:1, 4 ml) was added an alkyne (1.0 equiv) followed by sodium ascorbate (1.0 M solution in deionized water, 200 μL, 10 mol %), and copper (II) sulfate pentahydrate (6 mg in 100 μL of deionized water, 2 mol %). The reaction mixture was then treated with KHCO₃ (866 mg, 4.33 mmol) and left stirring for 1.5 h at room temperature before being poured onto water (25 ml) and extracted with EtOAc (2 × 25 ml). The combined organic phases were washed with saturated aqueous solution of NH₄Cl (30 ml), 10% aqueous solution of sodium tartrate (30 ml), and water (30 ml). The organic phase was dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give crude material, which was used in the next step without additional purification.

Individual Compounds

4-(5,5-Dimethyl-1,3-dioxan-2-yl)-2-methoxyphenol (2): The title compound was prepared following a literature procedure.^[39] Vanillin (4.56 g, 30 mmol), sulfamic acid (435 mg, 4.5 mmol, 0.15 equiv), and neopentyl glycol (3.55 g, 36 mmol, 1.2 equiv) were refluxed in dry toluene (25 ml) for 3 h. The reaction mixture was cooled to room temperature and poured into toluene/water (1:1, 300 ml). The phases were separated, and the organic phase was washed with water (150 ml), dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give crude material as a red oil, which was purified by flash chromatography (hexanes–EtOAc, 5:1 to 3:1) to give pure product as a white crystalline material (6.19 g, 87%).

¹H NMR (DMSO-*d*₆) δ 9.02 (s, 1H), 6.94 (d, *J* = 1.5 Hz, 1H), 6.84 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.75 (d, *J* = 8.1 Hz, 1H), 5.28 (s, 1H), 3.76 (s, 3H), 3.64 (d, *J* = 10.8 Hz, 2H), 3.58 (d, *J* = 10.8 Hz, 2H), 1.18 (s, 3H), 0.73 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 147.1, 146.8, 130.0, 118.9, 114.8, 110.1, 101.0, 76.5 (2C), 55.5, 29.7, 22.8, 21.4; HPLC purity 90%; MS (ESI+) *m/z* 239 (M + H⁺).

2-[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl]-5,5-dimethyl-1,3-dioxane (3): The title compound was prepared following a literature procedure.^[40] 4-(5,5-Dimethyl-1,3-dioxan-2-yl)-2-methoxyphenol (**2**) (4.76 g, 20 mmol, 1.0 equiv), 1,3-dichloro-4-fluorobenzene (6.60 g, 40 mmol, 2.0 equiv), and Cs₂CO₃ (13.04 g, 40 mmol, 2.0 equiv) were dissolved in dry DMF (50 ml) and stirred at 130 °C for 14 h. The reaction mixture was cooled to room temperature and poured into water (200 ml), and the products were extracted into EtOAc (3 × 100 ml). The combined organic phases were washed with water (100 ml), dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give crude material as a yellow solid, which was purified by re-crystallization from CH₂Cl₂/EtOAc to give the pure product as a white crystalline material (3.90 g, 51%).

¹H NMR (DMSO-*d*₆) δ 7.69 (s, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 7.20 (s, 1H), 7.06 – 7.01 (m, 2H), 6.68 (d, *J* = 8.6 Hz, 1H), 5.43 (s, 1H), 3.75 (s, 3H), 3.70 (d, *J* = 10.4 Hz, 2H), 3.64 (d, *J* = 10.4 Hz, 2H), 1.19 (s, 3H), 0.76 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 152.1, 150.3, 143.0, 136.8, 129.8, 128.4, 126.6, 123.1, 120.5, 119.0, 118.0, 111.2, 100.2, 76.5 (2C), 55.8, 29.8, 22.8, 21.4; HPLC purity 98.7%; MS (ESI+) *m/z* 383 (M+H⁺, ³⁵Cl₂).

4-(2,4-Dichlorophenoxy)-3-methoxybenzaldehyde (4): The title compound was prepared following a literature procedure.^[41] 2-[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl]-5,5-dimethyl-1,3-dioxane (**3**) (4.60 g, 12 mmol) was dissolved in wet acetone (100 ml), and pyridinium *p*-toluenesulfonate (1.15 g, 4.56 mmol, 0.38 equiv) was added. The reaction mixture was refluxed for 2.5 h before cooling to room temperature. The solvent was evaporated *in vacuo* and the residue dissolved in diethyl ether (100 ml). The organic phase was washed with saturated aqueous solution of NaHCO₃ (100 ml) and brine, dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give crude material as a pale-yellow oil (3.56 g, 100%). The crude material was of sufficient purity to be used directly in the next step.

¹H NMR (DMSO-*d*₆) δ 9.94 (s, 1H), 7.77 (d, *J* = 2.5 Hz, 1H), 7.63 (d, *J* = 1.7 Hz, 1H), 7.55 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.41 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.04 (overlapping d, *J* = 8.2 Hz, 1H), 7.02 (overlapping d, *J* = 8.8 Hz, 1H), 3.88 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 191.7, 150.4 (2C), 149.2, 133.2, 130.1, 128.8, 128.5, 124.8, 124.6, 120.9, 118.6, 112.1, 56.0; HPLC purity 98.3%.

[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl]methanol (5): The title compound was prepared following a literature procedure.^[42] 4-(2,4-Dichlorophenoxy)-3-methoxybenzaldehyde (**4**) (3.56 g, 12 mmol) was dissolved in dry MeOH (100 ml) at room temperature, and NaBH₄ (2.27 g, 60 mmol, 5 equiv) was added in portions. The reaction mixture was stirred at room temperature for 1.5 h before quenching with saturated aqueous solution of NH₄Cl (25 ml). The mixture was poured into saturated aqueous solution of NH₄Cl/EtOAc (1:1, 100 ml), and the products were extracted into EtOAc (2 × 100 ml). The combined organic phases were washed with water (100 ml), dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give crude material as a pale-yellow oil, which was purified by flash chromatography (hexanes–EtOAc, 5:1 to 3:1) to give the pure product as a pale-yellow oil (3.03 g, 84%).

¹H NMR (DMSO-*d*₆) δ 7.66 (d, *J* = 2.5 Hz, 1H), 7.28 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.15 (d, *J* = 1.5 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 1H), 6.94 (dd, *J* = 8.1, 1.7 Hz, 1H), 6.62 (d, *J* = 8.9 Hz, 1H), 5.27 (t, *J* = 5.7 Hz, 1H), 4.52 (d, *J* = 5.7 Hz, 2H), 3.74 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 152.5, 150.5, 141.2, 141.1, 129.6, 128.2, 126.1, 122.7, 121.0, 118.9, 117.4, 111.5, 62.5, 55.7; HPLC purity 98.9%; MS (ESI+) *m/z* 281 (M+H⁺–H₂O, ³⁵Cl₂).

4-(Bromomethyl)-1-(2,4-dichlorophenoxy)-2-methoxybenzene (6): The title compound was prepared following a modified literature procedure.^[43] [4-(2,4-Dichlorophenoxy)-3-

methoxyphenyl]methanol (**5**) (2.99 g, 10.0 mmol) was dissolved in dry THF (50 ml) followed by addition of carbon tetrabromide (4.97 g, 15.0 mmol, 1.5 equiv) and triphenylphosphine (3.93 g, 15.0 mmol, 1.5 equiv). The reaction mixture was stirred at room temperature for 14 h, then poured into hexane (250 ml) and vigorously stirred. The mixture was filtered through a silica pad followed by elution with hexane–EtOAc (1:1, 250 ml). The solution was concentrated *in vacuo* to give a yellow oil, which was purified by flash chromatography (hexanes, then hexanes–EtOAc 5:1) to give the pure product as a pale-yellow oil (3.00 g, 83%).

^1H NMR (DMSO- d_6) δ 7.69 (d, J = 2.6 Hz, 1H), 7.32 (dd, J = 8.8, 2.6 Hz, 1H), 7.30 (d, J = 1.7 Hz, 1H), 7.07 (overlapping dd, J = 8.2, 1.9 Hz, 1H), 6.99 (overlapping d, J = 8.2 Hz, 1H), 6.72 (d, J = 8.8 Hz, 1H), 4.72 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 151.8, 150.4, 143.0, 135.9, 129.8, 128.4, 126.8, 123.3, 122.2, 120.7, 118.3, 114.4, 55.8, 34.2; HPLC purity 91.9%; MS (ESI+) m/z 281 ($\text{M}^+ - \text{Br}$, $^{35}\text{Cl}_2$).

4-(Azidomethyl)-1-(2,4-dichlorophenoxy)-2-methoxybenzene (7): This compound was prepared following a literature procedure.^[44] To a stirred solution of 4-(bromomethyl)-1-(2,4-dichlorophenoxy)-2-methoxybenzene (**6**) (2.9 g, 8.0 mmol) in dry DMF (24 ml) was added at room temperature sodium azide (0.78 g, 12.0 mmol, 1.5 equiv). The reaction mixture was stirred at 110 °C for 7 h before cooling to room temperature and diluting with EtOAc (150 ml). The mixture was washed with saturated aqueous solution of NaHCO_3 (150 ml) and water (2×150 ml), dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to give the crude material as a pale-yellow oil, which was purified by flash chromatography (hexanes, then hexanes–EtOAc 5:1) to give the pure product as a pale-yellow oil (1.84 g, 71%).

^1H NMR (DMSO- d_6) δ 7.67 (d, J = 2.5 Hz, 1H), 7.30 (dd, J = 8.8, 2.6 Hz, 1H), 7.22 (d, J = 1.6 Hz, 1H), 7.05 (d, J = 8.1 Hz, 1H), 6.99 (dd, J = 8.1, 1.7 Hz, 1H), 6.70 (d, J = 8.8 Hz, 1H), 4.47 (s, 2H), 3.77 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 152.0, 150.7, 142.7, 133.7, 129.7, 128.3, 126.6, 123.2, 121.1, 120.9, 118.0, 113.6, 55.8, 53.3; HPLC purity 98.6%; MS (ESI+) m/z 281 ($\text{M}^+ - \text{N}_3$, $^{35}\text{Cl}_2$).

4-Butyl-1-[4-(2,4-dichlorophenoxy)-3-methoxybenzyl]-1H-1,2,3-triazole (8a): The title compound was prepared following the General Procedure A with 1-hexyne as the alkyne (pale-yellow oil, 165 mg, 86% on a 0.47 mmolar scale).

^1H NMR (DMSO- d_6) δ 7.94 (s, 1H), 7.69 (d, J = 2.5 Hz, 1H), 7.29 (dd, J = 8.9, 2.6 Hz, 1H), 7.20 (d, J = 1.8 Hz, 1H), 7.02 (d, J = 8.2 Hz, 1H), 6.86 (dd, J = 8.2, 1.9 Hz, 1H), 6.68 (d, J = 8.9 Hz, 1H), 5.54 (s, 2H), 3.74 (s, 3H), 2.61 (t, J = 7.6 Hz, 2H), 1.57 (dt, J = 15.1, 7.5 Hz, 2H), 1.31 (dq, J = 14.8, 7.4 Hz, 2H), 0.89 (t, J = 7.3 Hz, 3H); ^{13}C NMR (DMSO- d_6) δ 151.9, 150.6, 147.2, 142.7, 134.2, 129.8, 128.4, 126.7, 123.2, 121.9, 121.0, 120.6, 118.1, 113.3, 55.8, 52.3, 31.1, 24.6, 21.6, 13.6; HPLC purity 87.6%; MS (ESI+) m/z 406 ($\text{M} + \text{H}^+$, $^{35}\text{Cl}_2$).

1-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]-4-phenyl-1H-1,2,3-triazole (8b): The title compound was prepared following the General Procedure A with phenylacetylene as the alkyne (pale-yellow solid, 213 mg, 100%).

^1H NMR (DMSO- d_6) δ 8.66 (s, 1H), 7.86 (dd, J = 8.3, 1.7 Hz, 2H), 7.69 (d, J = 2.5 Hz, 1H), 7.44 (t, J = 7.6 Hz, 2H), 7.35 – 7.27 (m, 3H), 7.05 (d, J = 8.1 Hz, 1H), 6.95 (dd, J = 8.2, 1.9 Hz, 1H), 6.69 (d, J = 8.8 Hz, 1H), 5.65 (s, 2H), 3.77 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 151.9, 150.6, 146.6, 142.8, 133.8, 130.6, 129.7, 128.9 (2C), 128.4, 127.9, 126.7, 125.2 (2C), 123.2, 121.5, 121.1, 120.9, 118.1, 113.6, 55.9, 52.7; HPLC purity 90%; MS (ESI+) m/z 426 ($\text{M} + \text{H}^+$, $^{35}\text{Cl}_2$).

1-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]-4-(trimethylsilyl)-1H-1,2,3-triazole (8c):

The title compound was prepared following the General Procedure A with trimethylsilylacetylene as the alkyne (pale-yellow solid, 571 mg, 97% on a 1.5 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 8.24 (s, 1H), 7.69 (d, *J* = 2.5 Hz, 1H), 7.29 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.26 (d, *J* = 1.9 Hz, 1H), 7.02 (d, *J* = 8.2 Hz, 1H), 6.89 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.69 (d, *J* = 8.8 Hz, 1H), 5.60 (s, 2H), 3.75 (s, 3H), 0.26 (s, 9H); ¹³C NMR (DMSO-*d*₆) δ 151.9, 150.6, 145.1, 142.7, 134.1, 130.6, 129.8, 128.4, 126.7, 123.2, 121.0, 120.8, 118.2, 113.6, 55.8, 51.9, -1.0 (3C); HPLC purity 86.1%; MS (ESI+) *m/z* 422 (M+H⁺, ³⁵Cl₂).

2-[1-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]-1H-1,2,3-triazol-4-yl]ethanol (8d):

The title compound was prepared following the General Procedure A with 4-butyne-1-ol as the alkyne (yellow oil, 395 mg, 100%). Purification by preparative HPLC provided the pure material as pale-yellow oil (318 mg, 81% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 7.95 (s, 1H), 7.68 (d, *J* = 2.7 Hz, 1H), 7.29 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.23 (d, *J* = 1.9 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 1H), 6.88 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.69 (d, *J* = 8.8 Hz, 1H), 5.54 (s, 2H), 4.44 (s, 1H), 3.74 (s, 3H), 3.63 (t, *J* = 6.9 Hz, 2H), 2.77 (t, *J* = 6.9 Hz, 2H); ¹³C NMR (DMSO-*d*₆) δ 152.0, 150.6, 144.8, 142.7, 134.1, 129.8, 128.4, 126.7, 123.2, 122.6, 121.0, 120.8, 118.1, 113.5, 60.3, 55.9, 52.3, 29.2; HPLC purity 97.0%; MS (ESI+) *m/z* 394 (M+H⁺, ³⁵Cl₂).

5-[(4-Butyl-1H-1,2,3-triazol-1-yl)methyl]-2-(2,4-dichlorophenoxy)phenol (9a): The title compound was prepared following the General Procedure B with **8a** as the starting material. Purification by preparative HPLC provided the pure material as a white crystalline solid (84 mg, 43% on a 0.4 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 9.89 (s, 1H), 7.89 (s, 1H), 7.67 (d, *J* = 2.5 Hz, 1H), 7.29 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 6.85 (d, *J* = 2.0 Hz, 1H), 6.76 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.66 (d, *J* = 8.8 Hz, 1H), 5.72 (s, 2H), 2.61 (t, *J* = 7.7 Hz, 2H), 1.57 (quin, *J* = 7.6 Hz, 2H), 1.32 (sxt, *J* = 7.3 Hz, 2H), 0.89 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 152.2, 148.9, 147.2, 141.6, 134.2, 129.6, 128.2, 126.2, 123.0, 122.0, 121.6, 119.1, 117.9, 116.5, 52.1, 31.1, 24.7, 21.7, 13.7; HPLC purity 99.7%; MS (ESI+) *m/z* 392 (M+H⁺, ³⁵Cl₂); HRMS (ESI+) calcd for C₁₉H₂₀Cl₂N₃O₂ 392.0927 (M+H⁺, ³⁵Cl₂), found 392.0924.

2-(2,4-Dichlorophenoxy)-5-[(4-phenyl-1H-1,2,3-triazol-1-yl)methyl]phenol (9b): The title compound was prepared following the General Procedure B with **8b** as the starting material. Purification by preparative HPLC provided the pure material as a white crystalline solid (53 mg, 26%).

¹H NMR (DMSO-*d*₆) δ 9.91 (s, 1H), 8.65 (s, 1H), 7.87 – 7.85 (m, 2H), 7.67 (d, *J* = 2.6 Hz, 1H), 7.47 – 7.43 (m, 2H), 7.33 (tt, *J* = 7.4, 1.2 Hz, 1H), 7.29 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.01 (d, *J* = 8.2 Hz, 1H), 6.91 (d, *J* = 2.1 Hz, 1H), 6.84 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.67 (d, *J* = 8.9 Hz, 1H), 5.60 (s, 2H); ¹³C NMR (DMSO-*d*₆) δ 152.2, 149.0, 146.6, 141.7, 133.9, 130.6, 129.6, 128.9 (2C), 128.2, 127.9, 126.2, 125.1 (2C), 123.0, 121.7, 121.6, 119.2, 117.8, 116.5, 52.5; HPLC purity 98.4%; MS (ESI+) *m/z* 412 (M+H⁺, ³⁵Cl₂); HRMS (ESI+) calcd for C₂₁H₁₆Cl₂N₃O₂ 412.0614 (M+H⁺, ³⁵Cl₂), found 412.0596.

2-(2,4-Dichlorophenoxy)-5-(1H-1,2,3-triazol-1-ylmethyl)phenol (9c)

1-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]-1H-1,2,3-triazole: The title compound was prepared following a literature procedure.^[46] To a stirred solution of **8c** (0.5 mmol) in dry THF (3.0 ml) was added dropwise at room temperature *n*-Bu₄NF (1.50 ml of a 1.0 M

solution in THF, 1.50 mmol, 3.0 equiv) and stirring was continued at room temperature for 23 h. After this time, MeOH (3.0 ml) was added, and the solvents were evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel (hexanes, then hexanes–EtOAc 3:1) to give the pure product as a colorless oil (100 mg, 57%).

^1H NMR (DMSO- d_6) δ 8.23 (d, J = 1.0 Hz, 1H), 7.76 (d, J = 0.9 Hz, 1H), 7.69 (d, J = 2.5 Hz, 1H), 7.29 (dd, J = 8.8, 2.6 Hz, 1H), 7.23 (d, J = 1.9 Hz, 1H), 7.03 (d, J = 8.1 Hz, 1H), 6.87 (dd, J = 8.2, 2.0 Hz, 1H), 6.68 (d, J = 8.8 Hz, 1H), 5.62 (s, 2H), 3.74 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 152.0, 150.6, 142.7, 134.1, 133.6, 129.8, 128.4, 126.7, 125.0, 123.1, 121.1, 120.7, 118.1, 113.4, 55.8, 52.2; HPLC purity 98.4%; MS (ESI+) m/z 350 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$).

Compound **9c** was prepared following the General Procedure B with 1-[4-(2,4-dichlorophenoxy)-3-methoxybenzyl]-1*H*-1,2,3-triazole as the starting material. Purification by preparative HPLC provided the pure product as a white solid (32 mg, 33% on a 0.29 mmolar scale).

^1H NMR (DMSO) δ 9.90 (s, 1H), 8.19 (d, J = 0.8 Hz, 1H), 7.77 (d, J = 0.7 Hz, 1H), 7.67 (d, J = 2.5 Hz, 1H), 7.29 (dd, J = 8.9, 2.6 Hz, 1H), 6.98 (d, J = 8.2 Hz, 1H), 6.86 (d, J = 1.9 Hz, 1H), 6.77 (dd, J = 8.2, 2.0 Hz, 1H), 6.66 (d, J = 8.9 Hz, 1H), 5.57 (br s, 2H); ^{13}C NMR (DMSO) δ 152.2, 148.9, 141.6, 134.1, 133.6, 129.6, 128.2, 126.2, 125.1, 123.0, 121.6, 119.1, 117.9, 116.5, 52.0; HPLC purity 99.7%; MS (ESI+) m/z 336 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$); HRMS (ESI+) calcd for $\text{C}_{15}\text{H}_{12}\text{Cl}_2\text{N}_3\text{O}_2$ 336.0301 ($\text{M}+\text{H}^+$), found 336.0297.

3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzaldehyde (11): The title compound was prepared following a literature procedure.^[48] To a stirred solution of 4-chloro-2-methoxyphenol (2.38 g, 15.0 mmol) in dry DMF (40 ml) was added 3-chloro-4-fluorobenzaldehyde (2.38g, 15.0 mmol, 1.0 equiv) followed by Cs_2CO_3 (8.31 g, 25.5 mmol, 1.70 equiv). The mixture was stirred at 100 °C for 16 h under an Ar atmosphere, cooled to room temperature, diluted with EtOAc, and poured into water. The phases were separated, and the aqueous phase was extracted with EtOAc (3 $\times$ 100 ml). The combined organic phases were washed with water (3 $\times$ 100 ml), dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to give the crude material as a dark-green solid, which was subsequently purified by flash chromatography on silica gel (hexanes, then hexanes–EtOAc 3:1 to 3:2) to obtain the title compound as a pale-yellow solid (4.10 g, 92%).

^1H NMR (DMSO- d_6) δ 9.88 (s, 1H), 8.07 (d, J = 1.9 Hz, 1H), 7.77 (dd, J = 8.5, 2.0 Hz, 1H), 7.34 (d, J = 2.3 Hz, 1H), 7.25 (d, J = 8.6 Hz, 1H), 7.10 (dd, J = 8.5, 2.4 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 3.76 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 190.7, 157.6, 151.8, 140.7, 131.6 (2C), 130.9, 130.0, 123.4, 122.2, 121.1, 115.8, 114.2, 56.4; HPLC purity 93.0%.

[3-Chloro-4-(4-chloro-2-methoxyphenoxy)phenyl]methanol (12): The same procedure was applied as described for the synthesis of compound **5** to give the title compound as pale-yellow oil (2.9 g, 100% on a 9.7 mmolar scale).

^1H NMR (DMSO- d_6) δ 7.46 (s, 1H), 7.25 (d, J = 2.3 Hz, 1H), 7.19 (dd, J = 8.5, 1.9 Hz, 1H), 6.99 (dd, J = 8.6, 2.4 Hz, 1H), 6.92 (d, J = 8.5 Hz, 1H), 6.75 (d, J = 8.5 Hz, 1H), 5.27 (t, J = 5.7 Hz, 1H), 4.46 (d, J = 5.8 Hz, 2H), 3.79 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 151.3, 151.0, 142.9, 138.9, 129.0, 128.3, 126.4, 122.2, 121.1, 120.7, 117.4, 113.8, 61.8, 56.2; HPLC purity 98.4%.

4-(Bromomethyl)-2-chloro-1-(4-chloro-2-methoxyphenoxy)benzene (13): The same procedure was applied as described for the synthesis of compound **6** to give the title compound as a white crystalline material (2.8 g, 80% on a 9.6 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 7.65 (d, *J* = 2.1 Hz, 1H), 7.32 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.28 (d, *J* = 2.0 Hz, 1H), 7.05 – 7.04 (m, 2H), 6.69 (d, *J* = 8.5 Hz, 1H), 4.69 (s, 2H), 3.78 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 152.6, 151.6, 141.9, 133.8, 131.2, 129.8, 129.6, 122.3, 121.8, 120.8, 116.8, 113.9, 56.2, 33.1; HPLC purity 96.8%.

4-(Azidomethyl)-2-chloro-1-(4-chloro-2-methoxyphenoxy)benzene (14): The same procedure was applied as described for the synthesis of compound **7** to give the title compound as a yellow oil (1.34 g, 56% on a 7.45 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 7.58 (d, *J* = 2.1 Hz, 1H), 7.28 (dd, *J* = 2.4, 1.0 Hz, 1H), 7.25 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.04 – 7.03 (m, 2H), 6.75 (d, *J* = 8.3 Hz, 1H), 4.43 (s, 2H), 3.78 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 152.4, 151.5, 142.1, 131.5, 130.4, 129.6, 128.6, 122.1, 122.0, 120.8, 117.0, 113.9, 56.2, 52.3; HPLC purity 96.2%.

4-Butyl-1-[3-chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-1H-1,2,3-triazole (15a):

The title compound was prepared following the General Procedure A with 1-hexyne as the alkyne. Purification by flash chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂–MeOH 5:1 to 4:1) gave the pure product as a pale-yellow oil (324 mg, 80% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 7.91 (s, 1H), 7.51 (d, *J* = 2.1 Hz, 1H), 7.27 (m, 1H), 7.18 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.01 (m, 2H), 6.72 (d, *J* = 8.5 Hz, 1H), 5.49 (s, 2H), 3.76 (s, 3H), 2.59 (t, *J* = 7.6 Hz, 2H), 1.55 (dt, *J* = 15.2, 7.5 Hz, 2H), 1.30 (sxt, *J* = 7.4 Hz, 2H), 0.88 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 152.4, 151.6, 147.3, 142.1, 132.0, 130.0 (2C), 128.2, 122.1 (2C), 121.9, 120.8, 117.1, 113.9, 56.2, 51.4, 31.0, 24.6, 21.6, 13.7; HPLC purity 98.0%.

1-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-4-phenyl-1H-1,2,3-triazole (15b):

The title compound was prepared following the General Procedure A with phenylacetylene as the alkyne. Purification by flash chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂–MeOH 5:1 to 4:1) gave the pure product as pale-yellow oil (343 mg, 80% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 8.65 (s, 1H), 7.84 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.61 (d, *J* = 2.1 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.33 (tt, *J* = 7.4, 1.2 Hz, 1H), 7.27 (overlapping t, *J* = 1.1 Hz, 1H), 7.25 (overlapping dd, *J* = 8.5, 2.1 Hz, 1H), 7.01 (d, *J* = 1.3 Hz, 2H), 6.74 (d, *J* = 8.5 Hz, 1H), 5.61 (s, 2H), 3.76 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 152.5, 151.6, 146.7, 142.0, 131.6, 130.6, 130.2, 129.7, 128.9 (2C), 128.4, 127.9, 125.2 (2C), 122.2, 122.1, 121.5, 120.8, 117.1, 113.9, 56.2, 51.8; HPLC purity 96.0%.

1-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-4-(trimethylsilyl)-1H-1,2,3-triazole (15c):

The title compound was prepared following the General Procedure A with trimethylsilylacetylene as the alkyne. Purification by flash chromatography on silica gel (hexanes, then hexanes–EtOAc 3:1 to 3:2) gave the pure product as pale-yellow oil (319 mg, 82% on a 0.92 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 8.22 (s, 1H), 7.57 (d, *J* = 2.1 Hz, 1H), 7.27 (t, *J* = 1.2 Hz, 1H), 7.20 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.02 (d, *J* = 1.2 Hz, 2H), 6.71 (d, *J* = 8.5 Hz, 1H), 5.55 (s, 2H), 3.76 (s, 3H), 0.24 (s, 9H); ¹³C NMR (DMSO-*d*₆) δ 152.5, 151.6, 145.2, 142.0, 131.8, 130.5, 130.3, 129.7, 128.5, 122.1, 122.0, 120.8, 117.0, 113.9, 56.2, 51.0, –1.0 (3C); HPLC purity 96.3%; MS (ESI⁺) *m/z* 422 (M+H⁺, ³⁵Cl₂).

2-[4-[(4-Butyl-1H-1,2,3-triazol-1-yl)methyl]-2-chlorophenoxy]-5-chlorophenol (16a):

The title compound was prepared following the General Procedure C with **15a** as the starting material. Purification by flash chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂–

MeOH 5:1 to 4:1) followed by preparative HPLC gave the pure product as a white crystalline solid (142 mg, 48% on a 0.76 mmolar scale).

^1H NMR (DMSO- d_6) δ 10.22 (br s, 1H), 7.91 (s, 1H), 7.50 (d, J = 2.1 Hz, 1H), 7.18 (dd, J = 8.6, 2.1 Hz, 1H), 7.00 (d, J = 2.4 Hz, 1H), 6.96 (d, J = 8.6 Hz, 1H), 6.86 (dd, J = 8.6, 2.5 Hz, 1H), 6.71 (d, J = 8.5 Hz, 1H), 5.49 (s, 2H), 2.59 (t, J = 7.6 Hz, 2H), 1.55 (dt, J = 15.1, 7.5 Hz, 2H), 1.31 (sxt, J = 7.4 Hz, 2H), 0.88 (t, J = 7.3 Hz, 3H); ^{13}C NMR (DMSO- d_6) δ 152.6, 149.9, 147.3, 141.2, 131.7, 130.0, 129.1, 128.1, 122.6, 122.0, 121.8, 119.4, 117.0 (2C), 51.4, 31.0, 24.6, 21.6, 13.6; HPLC purity 99.7%; HRMS (ESI+) calcd for $\text{C}_{19}\text{H}_{20}\text{Cl}_2\text{N}_3\text{O}_2$ 392.0927 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$), found 392.0932.

5-Chloro-2-[2-chloro-4-[(4-phenyl-1H-1,2,3-triazol-1-yl)methyl]phenoxy]phenol (16b):

The title compound was prepared following the General Procedure C with **15b** as the starting material. Purification by flash chromatography on silica gel (CH_2Cl_2 , then CH_2Cl_2 –MeOH 5:1) followed by preparative HPLC gave the pure product as a white crystalline solid (200 mg, 63% on a 0.77 mmolar scale).

^1H NMR (DMSO- d_6) δ 10.23 (s, 1H), 8.65 (s, 1H), 7.84 (dd, J = 8.3, 1.3 Hz, 2H), 7.60 (d, J = 2.1 Hz, 1H), 7.44 (t, J = 7.6 Hz, 2H), 7.33 (tt, J = 7.4, 1.1 Hz, 1H), 7.26 (dd, J = 8.5, 2.1 Hz, 1H), 7.00 (d, J = 2.5 Hz, 1H), 6.96 (d, J = 8.6 Hz, 1H), 6.86 (dd, J = 8.5, 2.4 Hz, 1H), 6.74 (d, J = 8.5 Hz, 1H), 5.61 (s, 2H); ^{13}C NMR (DMSO- d_6) δ 152.7, 149.9, 146.7, 141.2, 131.3, 130.6, 130.1, 129.1, 128.9 (2C), 128.3, 127.9, 125.2 (2C), 122.6, 122.1, 121.5, 119.4, 117.1, 117.0, 51.8; HPLC purity 98.6%; HRMS (ESI+) calcd for $\text{C}_{21}\text{H}_{16}\text{Cl}_2\text{N}_3\text{O}_2$ 412.0614 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$), found 412.0622.

5-Chloro-2-[2-chloro-4-[(1H-1,2,3-triazol-1-yl)methyl]phenoxy]phenol (16c)

1-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-1H-1,2,3-triazole: The same procedure was applied as described for the synthesis of compound **9c**. Purification by flash chromatography on silica gel (CH_2Cl_2 , then CH_2Cl_2 –MeOH 5:1 to 4:1) gave the pure product as pale-yellow oil (190 mg, 76% on a 0.71 mmolar scale).

^1H NMR (DMSO- d_6) δ 8.21 (d, J = 1.0 Hz, 1H), 7.75 (d, J = 1.0 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.27 (dd, J = 1.5, 0.9 Hz, 1H), 7.19 (dd, J = 8.5, 2.2 Hz, 1H), 7.01 (m, 2H), 6.72 (d, J = 8.5 Hz, 1H), 5.58 (s, 2H), 3.76 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 152.4, 151.6, 142.1, 133.6, 131.9, 130.1, 129.7, 128.3, 125.0, 122.1 (2C), 120.8, 117.1, 113.9, 56.3, 51.4; HPLC purity 96.5%.

Compound **16c** was prepared following the General Procedure C with 1-[3-chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-1H-1,2,3-triazole as the starting material. Purification by flash chromatography on silica gel (CH_2Cl_2 , then CH_2Cl_2 –MeOH 5:1 to 4:1) followed by preparative HPLC gave the pure product as a white solid (111 mg, 65% on a 0.51 mmolar scale).

^1H NMR (DMSO- d_6) δ 10.23 (s, 1H), 8.21 (d, J = 0.9 Hz, 1H), 7.74 (d, J = 0.9 Hz, 1H), 7.52 (d, J = 2.1 Hz, 1H), 7.20 (dd, J = 8.5, 2.1 Hz, 1H), 7.00 (d, J = 2.5 Hz, 1H), 6.96 (d, J = 8.6 Hz, 1H), 6.86 (dd, J = 8.6, 2.5 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 5.58 (s, 2H); ^{13}C NMR (DMSO- d_6) δ 146.7, 143.9, 135.2, 127.6, 125.6, 124.0, 123.1, 122.2, 118.9, 116.6, 116.0, 113.4, 111.0 (2C), 45.4; HPLC purity 99.6%; HRMS (ESI+) calcd for $\text{C}_{15}\text{H}_{12}\text{Cl}_2\text{N}_3\text{O}_2$ 336.0301 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$), found 336.0309.

(E)-1-[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl]-N-hydroxymethanimine (17): The title compound was prepared following a literature procedure.^[45] To a solution of aldehyde **4** (2.08 g, 7.0 mmol) in a 1:1:2 mixture of H_2O /EtOH/ice (8.0 ml) was added at room

temperature hydroxylamine hydrochloride (0.49 g, 7.0 mmol, 1.0 equiv), followed by dropwise addition of NaOH (1.40 ml of a 50% solution in H₂O, 17.5 mmol, 2.5 equiv), while keeping the temperature below 30 °C. After stirring at room temperature for 75 min, the reaction mixture was washed with diethyl ether (30 ml). The aqueous phase was acidified to pH 6 by addition of concentrated HCl while keeping the temperature below 30 °C, and extracted with Et₂O (2 × 30 ml). The combined organic phases were dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give crude material as a milky white solid, which was of sufficient purity to be used directly in the next step (1.96 g, 90%).

¹H NMR (DMSO-*d*₆) δ 11.24 (s, 1H), 8.14 (s, 1H), 7.71 (d, *J* = 2.5 Hz, 1H), 7.41 (d, *J* = 1.7 Hz, 1H), 6.32 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.19 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 3.78 (s, 3H); HPLC purity 85.8%.

4-(2,4-Dichlorophenoxy)-*N*-hydroxy-3-methoxybenzenecarboximidoyl Chloride (18):

The title compound was prepared following a literature procedure.^[45] Oxime **17** (1.95 g, 6.25 mmol) was dissolved in dry DMF (7 ml), and *N*-chlorosuccinimide (NCS; 0.868 g, 6.5 mmol) was added portionwise at room temperature. The reaction mixture was stirred at room temperature for 1.5 h before being poured into water and extracted with Et₂O (2 × 50 ml). The combined organic phases were washed with brine (50 ml), dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give crude material as pale-red oil, which was used directly in the next step (2.17 g, 100%).

¹H NMR (DMSO-*d*₆) δ 12.42 (s, 1H), 7.73 (d, *J* = 2.5 Hz, 1H), 7.51 (d, *J* = 1.9 Hz, 1H), 7.40 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.35 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.05 (d, *J* = 8.5 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 3.82 (s, 3H); HPLC purity 75.3%.

5-Butyl-3-[4-(2,4-dichlorophenoxy)-3-methoxyphenyl]-1,2-oxazole (19a), **3-[4-(2,4-dichlorophenoxy)-3-methoxyphenyl]-5-phenyl-1,2-oxazole (19b)**, and **3-[4-(2,4-dichlorophenoxy)-3-methoxyphenyl]-5-(trimethylsilyl)-1,2-oxazole (19c)** were prepared following the General Procedure D using benzenecarboximidoyl chloride (**18**) and the following alkynes: 1-hexyne to give **19a** (yellow oil, 635 mg, 81%); phenylacetylene to give **19b** (yellow oil, 810 mg, 98%); trimethylsilylacetylene to give **19c** (yellow oil, 773 mg, 95%).

5-(5-Butyl-1,2-oxazol-3-yl)-2-(2,4-dichlorophenoxy)phenol (20a): The title compound was prepared following the General Procedure B with **19a** as the starting material. The crude material was purified by preparative HPLC to provide the pure product as a yellow solid (136 mg, 36% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 10.08 (s, 1H), 7.71 (d, *J* = 2.1 Hz, 1H), 7.48 (br s, 1H), 7.33 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.29 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.81 (d, *J* = 8.8 Hz, 1H), 6.72 (s, 1H), 2.78 (t, *J* = 7.5 Hz, 2H), 1.66 (quin, *J* = 7.5 Hz, 2H), 1.36 (sxt, *J* = 7.4 Hz, 2H), 0.92 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 174.1, 161.2, 151.9, 149.0, 143.6, 129.7, 128.4, 126.7, 126.5, 123.4, 121.4, 118.6 (2C), 114.9, 99.2, 29.0, 25.6, 21.6, 13.5; HPLC purity 98.0%; MS (ESI+) *m/z* 378 (M+H⁺, ³⁵Cl₂); HRMS (ESI+) calcd for C₁₉H₁₈Cl₂NO₃ 378.0658 (M+H⁺, ³⁵Cl₂), found 378.0644.

2-(2,4-Dichlorophenoxy)-5-(5-phenyl-1,2-oxazol-3-yl)phenol (20b): The title compound was prepared following the General Procedure B with **19b** as the starting material. The crude material was purified by preparative HPLC to provide the pure product as pale-yellow solid (118 mg, 30% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 10.16 (s, 1H), 7.93 (dd, *J* = 8.3, 1.5 Hz, 2H), 7.72 (d, *J* = 2.5 Hz, 1H), 7.60–7.54 (m, 5H), 7.38 (overlapping dd, *J* = 8.3, 2.0 Hz, 1H), 7.35 (overlapping dd, *J* = 8.8, 2.5 Hz, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 6.85 (d, *J* = 8.8 Hz, 1H); ¹³C NMR (DMSO-*d*₆) δ 169.7, 162.1, 151.9, 149.1, 143.9, 130.5, 129.8, 129.3 (2C), 128.4, 126.8 (2C), 126.1, 125.6 (2C), 123.5, 121.5, 118.7 (2C), 115.0, 98.6; HPLC purity 97.4%; MS (ESI+) *m/z* 398 (M+H⁺, ³⁵Cl₂); HRMS (ESI+) calcd for C₂₁H₁₄Cl₂NO₃ 398.0345 (M+H⁺, ³⁵Cl₂), found 398.0330.

2-(2,4-Dichlorophenoxy)-5-[5-(trimethylsilyl)-1,2-oxazol-3-yl]phenol (20c): The title compound was prepared following the General Procedure B with **19c** as the starting material. The crude material was purified by preparative HPLC to provide the pure product as a yellow crystalline solid (205 mg, 52% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 10.08 (s, 1H), 7.71 (d, *J* = 2.5 Hz, 1H), 7.52 (d, *J* = 2.0 Hz, 1H), 7.35 (overlapping dd, *J* = 8.8, 2.5 Hz, 1H), 7.32 (overlapping dd, *J* = 8.3, 2.0 Hz, 1H), 7.22 (br s, 1H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.82 (d, *J* = 8.8 Hz, 1H), 0.36 (s, 9H); ¹³C NMR (DMSO-*d*₆) δ 178.5, 159.8, 151.9, 149.0, 143.6, 129.7, 128.4, 126.8, 126.2, 123.4, 121.4, 118.8, 118.6, 115.3, 111.6, -2.0 (3C); HPLC purity 99.3%; MS (ESI+) *m/z* 394 (M+H⁺, ³⁵Cl₂); HRMS (ESI+) calcd for C₁₈H₁₈Cl₂NO₃Si 394.0428 (M+H⁺, ³⁵Cl₂), found 394.0431.

(E)-1-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)phenyl]-N-hydroxymethanimine (21): The title compound was prepared following a literature procedure.⁴⁵ The product was obtained as a pale-yellow solid in a yield of 0.70g (90% on a 2.5 mmolar scale) and was used directly in the next step.

¹H NMR (DMSO-*d*₆) δ 11.28 (s, 1H), 8.10 (s, 1H), 7.73 (d, *J* = 1.8 Hz, 1H), 7.47 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.29 (dd, *J* = 2.1 Hz, 1H), 7.08 (d, *J* = 8.5 Hz, 1H), 7.04 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.73 (d, *J* = 8.6 Hz, 1H), 3.78 (s, 3H).

3-Chloro-4-(4-chloro-2-methoxyphenoxy)-N-hydroxybenzenecarboximidoyl Chloride (22): The title compound was prepared following a literature procedure.⁴⁵ The product was obtained as a yellow dense oil in a yield of 0.78 g (100% on a 2.25 mmolar scale) and was used directly in the next step.

¹H NMR (DMSO-*d*₆) δ 12.46 (s, 1H), 7.86 (d, *J* = 2.3 Hz, 1H), 7.64 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.31 (d, *J* = 2.3 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 7.06 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.75 (d, *J* = 8.8 Hz, 1H), 3.77 (s, 3H).

5-Butyl-3-[3-chloro-4-(4-chloro-2-methoxyphenoxy)phenyl]-1,2-oxazole (23a) and 3-[3-chloro-4-(4-chloro-2-methoxyphenoxy)phenyl]-5-phenyl-1,2-oxazole (23b) were prepared following the General Procedure D with benzenecarboximidoyl chloride (**22**) and the following alkynes as the starting materials: 1-hexyne to give **23a** (yellow oil, 302 mg, 70% on a 1.1 mmolar scale); phenylacetylene to give **23b** (yellow solid, 400 mg, 88% on a 1.1 mmolar scale).

2-[4-(5-Butyl-1,2-oxazol-3-yl)-2-chlorophenoxy]-5-chlorophenol (24a): The title compound was prepared following the General Procedure B with **23a** as the starting material. The crude material was purified by preparative HPLC to provide the pure product as a white crystalline solid (80 mg, 27% on a 0.77 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 10.33 (s, 1H), 8.00 (d, *J* = 2.1 Hz, 1H), 7.76 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 7.03 (d, *J* = 2.5 Hz, 1H), 6.91 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.84

(br s, 1H), 6.79 (d, $J = 8.6$ Hz, 1H), 2.78 (t, $J = 7.5$ Hz, 2H), 1.66 (quin, $J = 7.5$ Hz, 2H), 1.37 (sxt, $J = 7.4$ Hz, 2H), 0.92 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (DMSO- d_6) δ 174.3, 160.3, 154.2, 150.0, 140.8, 129.5, 128.4, 126.5, 126.5, 124.2, 123.2, 122.4, 119.6, 117.1, 116.8, 99.2, 30.0, 25.6, 21.6, 13.6; HPLC purity 99.6%; MS (ESI+) m/z 378 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$); HRMS (ESI+) calcd for $\text{C}_{19}\text{H}_{18}\text{Cl}_2\text{NO}_3$ 378.0658 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$), found 378.0663.

5-Chloro-2-[2-chloro-4-(5-phenyl-1,2-oxazol-3-yl)phenoxy]phenol (24b): The title compound was prepared following the General Procedure B with **23b** as the starting material. The crude material was purified by preparative HPLC to provide the pure product as pale-yellow crystalline solid (126 mg, 33% on a 0.97 mmolar scale).

^1H NMR (DMSO- d_6) δ 10.36 (s, 1H), 8.07 (d, $J = 2.1$ Hz, 1H), 7.90 (dd, $J = 8.1$, 1.4 Hz, 2H), 7.80 (d, $J = 8.6$, 2.0 Hz, 1H), 7.64 (s, 1H), 7.60–7.51 (m, 3H), 7.11 (d, $J = 8.6$ Hz, 1H), 7.05 (d, $J = 2.4$ Hz, 1H), 6.92 (dd, $J = 8.5$, 2.5 Hz, 1H), 6.85 (d, $J = 8.6$ Hz, 1H); ^{13}C NMR (DMSO- d_6) δ 169.8, 161.2, 154.4, 150.1, 140.8, 130.6, 129.6, 129.3 (2C), 128.5, 126.7, 126.6, 125.5 (2C), 123.8, 123.2, 122.5, 119.6, 117.2, 116.9, 98.6; HPLC purity 99.2%; MS (ESI+) m/z 398 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$); HRMS (ESI+) calcd for $\text{C}_{21}\text{H}_{14}\text{Cl}_2\text{NO}_3$ 398.0345 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$), found 398.0346.

4-(2,4-Dichlorophenoxy)-3-methoxybenzonitrile (26): The title compound was prepared following a literature procedure.^[40] 4-Fluoro-3-methoxybenzonitrile (755 mg, 5.0 mmol), 2,4-dichlorophenol (978 mg, 6.0 mmol, 1.2 equiv), and Cs_2CO_3 (1.956 g, 6.0 mmol, 1.2 equiv) were suspended in dry DMF (10 ml) and stirred at 120 °C for 21 h under an Ar atmosphere. The reaction mixture was cooled to room temperature, poured onto water (75 ml), and extracted with EtOAc (2 $\times$ 75 ml). The combined organic phases were washed with water (75 ml) and brine (75 ml), dried over Na_2SO_4 , filtered, and concentrated *in vacuo* to give crude material as a red oil, which was purified by flash chromatography on silica gel (hexanes, then hexanes–EtOAc 5:1 to 3:1) to give the pure product as pale-yellow oil (382 mg, 65%).

^1H NMR (DMSO- d_6) δ 7.76 (d, $J = 2.5$ Hz, 1H), 7.68 (d, $J = 1.9$ Hz, 1H), 7.42 (overlapping dd, $J = 8.3$, 1.9 Hz, 1H), 7.40 (overlapping dd, $J = 8.8$, 2.5 Hz, 1H), 7.01 (overlapping d, $J = 8.8$ Hz, 1H), 7.00 (overlapping d, $J = 8.3$ Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 150.2 (2C), 148.1, 130.1, 128.8, 128.6, 126.1, 124.7, 120.9, 119.3, 118.5, 116.8, 107.3, 56.4; HPLC purity 95.5%.

4-(2,4-Dichlorophenoxy)-3-hydroxybenzonitrile (27): The title compound was prepared following the General Procedure B with **26** as the starting material. The crude material was purified by flash chromatography on silica gel (hexanes, then hexanes–EtOAc 5:1 to 3:1) to give the pure product as a pale-yellow oil (230 mg, 82% on a 1.0 mmolar scale).

^1H NMR (DMSO- d_6) δ 10.61 (br s, 1H), 7.75 (d, $J = 2.4$ Hz, 1H), 7.39 (dd, $J = 8.8$, 2.5 Hz, 1H), 7.31 (d, $J = 1.8$ Hz, 1H), 7.27 (dd, $J = 8.3$, 1.8 Hz, 1H), 6.99 (overlapping d, $J = 8.4$ Hz, 1H), 6.97 (overlapping d, $J = 8.9$ Hz, 1H); ^{13}C NMR (DMSO- d_6) δ 150.6, 148.9, 147.2, 130.0, 128.7, 128.1, 124.5, 124.3, 120.4, 120.3, 120.2, 118.5, 107.3; HPLC purity 99.3%; MS (ESI+) m/z 320 ($\text{M} + \text{Na}^+ + \text{H}_2\text{O}$, $^{35}\text{Cl}_2$). HRMS (ESI–) calcd for $\text{C}_{13}\text{H}_6\text{Cl}_2\text{NO}_2$ 277.9781 ($\text{M}-\text{H}^+$, $^{35}\text{Cl}_2$), found 277.9771.

4-(2,4-Dichlorophenoxy)-3-methoxybenzoic Acid (28): The title compound was prepared following a literature procedure.^[37] Nitrile **26** (1.47 g, 5.0 mmol) was dissolved in ethanol (16 ml), and NaOH (2.60 ml of a 25% aqueous solution) was added. The reaction mixture was refluxed for 21 h, cooled to room temperature, and acidified with 6.0 N HCl to give a white precipitate, which was filtered off, washed with water, and dried. This crude material

was purified by flash chromatography on silica gel (hexanes–EtOAc 3:1 to 2:3) to give the pure product as a white solid (1.05 g, 67%).

^1H NMR (DMSO- d_6) δ 13.03 (br s, 1H), 7.76 (d, J = 2.5 Hz, 1H), 7.65 (d, J = 1.9 Hz, 1H), 7.56 (dd, J = 8.3, 1.9 Hz, 1H), 7.38 (dd, J = 8.8, 2.5 Hz, 1H), 6.99 (d, J = 8.2 Hz, 1H), 6.92 (d, J = 8.8 Hz, 1H), 3.84 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 166.8, 151.0, 150.0, 147.6, 130.1, 128.8, 128.0, 127.8, 124.4, 123.0, 120.2, 119.1, 113.8, 56.0; HPLC purity 96.0%; MS (ESI $^-$) m/z 311 (M–H $^+$).

4-(2,4-Dichlorophenoxy)-3-methoxy-*N*-phenylbenzamide (29a): The title compound was prepared following a literature procedure.^[50] The crude product was purified by flash chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂–MeOH 10:1) to give the pure product as pale-yellow oil (220 mg, 81% on a 0.70 mmolar scale).

^1H NMR (DMSO- d_6) δ 10.23 (br s, 1H), 7.77 – 7.74 (m, 3H), 7.72 (d, J = 2.0 Hz, 1H), 7.61 (dd, J = 8.3, 2.0 Hz, 1H), 7.39 – 7.34 (m, 3H), 7.11 (tt, J = 7.4, 1.2 Hz, 1H), 7.10 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 8.8 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 164.5, 151.3, 150.0, 146.1, 139.0, 132.2, 130.0, 128.6 (3C), 127.5, 123.9, 123.8, 121.0, 120.5 (2C), 119.5, 119.3, 112.8, 56.0; HPLC purity 84.7%; MS (ESI $^+$) m/z 388 (M+H $^+$, $^{35}\text{Cl}_2$).

4-(2,4-Dichlorophenoxy)-3-methoxy-*N*, *N*-dibutylbenzamide (29b): The title compound was prepared following a literature procedure.^[50] The crude material was purified by flash chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂–MeOH 10:1 to 5:1) to give the pure product as a colorless oil (296 mg, 100% on a 0.70 mmolar scale).

^1H NMR (DMSO- d_6) δ 7.71 (d, J = 2.5 Hz, 1H), 7.33 (dd, J = 8.8, 2.7 Hz, 1H), 7.11 (d, J = 1.9 Hz, 1H), 7.04 (d, J = 8.1 Hz, 1H), 6.91 (dd, J = 8.1, 1.9 Hz, 1H), 6.76 (d, J = 8.8 Hz, 1H), 3.83 (s, 3H), 3.39 (br s, 2H), 3.17 (br s, 2H), 1.57 – 1.49 (m, 4H), 1.32 (br s, 2H), 1.11 (br s, 2H), 0.93 (br s, 3H), 0.75 (br s, 3H); ^{13}C NMR (DMSO- d_6) δ 169.5, 151.8, 150.3, 143.4, 135.0, 129.8, 128.4, 127.0, 123.4, 120.4, 119.2, 118.4, 111.7, 56.0, 48.1, 43.9, 30.2, 29.3, 19.8, 19.2, 13.8, 13.4; HPLC purity 94.4%; MS (ESI $^+$) m/z 424 (M+H $^+$, $^{35}\text{Cl}_2$).

[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl](morpholin-4-yl)methanone (29c): The title compound was prepared following a literature procedure.^[50] The crude material was purified by flash chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂–MeOH 7:1 to 4:1) to give the pure product as a colorless oil (296 mg, 77% on a 1.0 mmolar scale).

^1H NMR (DMSO- d_6) δ 7.72 (d, J = 2.5 Hz, 1H), 7.33 (dd, J = 8.8, 2.6 Hz, 1H), 7.21 (d, J = 1.7 Hz, 1H), 7.04 (d, J = 8.1 Hz, 1H), 7.00 (dd, J = 8.1, 1.7 Hz, 1H), 6.82 (d, J = 8.8 Hz, 1H), 3.79 (s, 3H); 3.60 – 3.38 (m, 8H); ^{13}C NMR (DMSO- d_6) δ 168.3, 151.6, 150.3, 144.0, 133.2, 129.8, 128.5, 127.1, 123.5, 120.2, 119.9, 118.8, 112.5, 66.1 (2C), 56.0; HPLC purity 91.0%; MS (ESI $^+$) m/z 382 (M+H $^+$, $^{35}\text{Cl}_2$).

4-(2,4-Dichlorophenoxy)-*N*-hexyl-3-methoxybenzamide (29d): The title compound was prepared following a literature procedure.^[50] The crude material was purified by flash chromatography on silica gel (hexanes, then hexanes–EtOAc 3:1 to 3:2) to provide the product as a colorless oil (199 mg, 42% on a 1.2 mmolar scale), which contained 16% (by HPLC) of the starting material, the corresponding carboxylic acid.

^1H NMR (DMSO- d_6) δ 8.47 (t, J = 5.8 Hz, 1H), 7.73 (d, J = 2.5 Hz, 1H), 7.62 (d, J = 1.9 Hz, 1H), 7.47 (dd, J = 8.3, 1.9 Hz, 1H), 7.34 (dd, J = 8.8, 2.6 Hz, 1H), 7.03 (d, J = 8.3 Hz, 1H), 6.80 (d, J = 8.8 Hz, 1H), 3.82 (s, 3H), 3.25 (q, J = 6.9 Hz, 2H), 1.55 – 1.48 (m, 2H), 1.33 – 1.28 (m, 6H), 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR (DMSO- d_6) δ 165.2, 151.5, 150.1,

145.6, 132.2, 123.0, 128.6, 127.4, 123.8, 120.4, 119.7, 119.1, 112.3, 56.0, 31.1 (2C), 29.2, 26.2, 22.1, 14.0; HPLC purity 83.4%.

4-(2,4-Dichlorophenoxy)-3-hydroxy-N-phenylbenzamide (30a): The title compound was prepared following the General Procedure B with **29a** as the starting material. The crude material was purified by preparative HPLC to give the pure product as a white solid (103 mg, 51% on a 0.54 mmolar scale). ^1H NMR (DMSO) δ 10.20 (s, 1H), 10.12 (s, 1H), 7.76 (br d, J = 7.6 Hz, 2H), 7.73 (d, J = 2.6 Hz, 1H), 7.56 (d, J = 2.1 Hz, 1H), 7.46 (dd, J = 8.4, 2.1 Hz, 1H), 7.38 – 7.32 (m, 3H), 7.11 – 7.05 (m, 2H), 6.83 (d, J = 8.8 Hz, 1H); ^{13}C NMR (DMSO) δ 164.8, 151.7, 148.3, 145.0, 139.2, 132.5, 129.8, 128.6 (2C), 128.4, 127.0, 123.7, 123.6, 120.3 (2C), 120.2, 119.3, 118.9, 117.0; HPLC purity 100%; MS (ESI+) m/z 374 (M + H⁺, $^{35}\text{Cl}_2$); HRMS (ESI+) calcd for C₁₉H₁₄Cl₂NO₃ 374.0345 (M + H⁺, $^{35}\text{Cl}_2$), found 374.0334.

4-(2,4-Dichlorophenoxy)-3-hydroxy-N,N-dibutylbenzamide (30b): The title compound was prepared following the General Procedure B with **29b** as the starting material. The crude material was purified by preparative HPLC to give the pure product as an off-white solid (203 mg, 70% on a 0.70 mmolar scale).

^1H NMR (DMSO- d_6) δ 10.03 (s, 1H), 7.69 (d, J = 2.5 Hz, 1H), 7.32 (dd, J = 8.9, 2.6 Hz, 1H), 7.00 (d, J = 8.1 Hz, 1H), 6.92 (d, J = 1.9 Hz, 1H), 6.77 (overlapping dd, J = 8.1, 1.9 Hz, 1H), 6.74 (overlapping d, J = 8.9 Hz, 1H), 3.33 (br s, 2H), 3.17 (br s, 2H), 1.53 – 1.49 (m, 4H), 1.31 (br s, 2H), 1.12 (br s, 2H), 0.92 (br s, 3H), 0.75 (br s, 3H); ^{13}C NMR (DMSO- d_6) δ 169.6, 152.1, 148.6, 142.3, 135.0, 129.7, 128.3, 126.6, 123.2, 121.1, 118.2, 117.8, 115.4, 48.0, 43.7, 30.2, 29.2, 19.7, 19.2, 13.8, 13.4; HPLC purity 100%; MS (ESI+) m/z 410 (M + H⁺, $^{35}\text{Cl}_2$); HRMS (ESI+) calcd for C₂₁H₂₆Cl₂NO₃ 410.1284 (M + H⁺, $^{35}\text{Cl}_2$), found 410.1272.

[4-(2,4-Dichlorophenoxy)-3-hydroxyphenyl](morpholin-4-yl)methanone (30c): The title compound was prepared following the General Procedure B with **29c** as the starting material. The crude material was purified by preparative HPLC to give the pure product as a white solid (100 mg, 36% on a 0.75 mmolar scale).

^1H NMR (DMSO- d_6) δ 10.07 (br s, 1H), 7.70 (d, J = 2.5 Hz, 1H), 7.33 (dd, J = 8.9, 2.6 Hz, 1H), 7.00 – 6.98 (m, 2H), 6.86 (dd, J = 8.2, 2.0 Hz, 1H), 6.80 (d, J = 8.9 Hz, 1H), 3.60 – 3.48 (m, 8H); ^{13}C NMR (DMSO- d_6) δ 168.4, 151.9, 148.6, 143.0, 133.0, 129.7, 128.3, 126.7, 123.4, 120.8, 118.6, 118.5, 116.2, 66.1 (2C), 47.5 (2C); HPLC purity 97.6%; MS (ESI+) m/z 368 (M + H⁺, $^{35}\text{Cl}_2$); HRMS (ESI+) calcd for C₁₇H₁₆Cl₂NO₄ 368.0451 (M + H⁺, $^{35}\text{Cl}_2$), found 368.0445.

4-(2,4-Dichlorophenoxy)-N-hexyl-3-hydroxybenzamide (30d): The title compound was prepared following the General Procedure C with **29d** as the starting material. The crude material was purified by preparative HPLC to give the pure product as a white crystalline solid (129 mg, 69% on a 0.49 mmolar scale).

^1H NMR (DMSO- d_6) δ 9.98 (s, 1H), 8.37 (t, J = 5.6 Hz, 1H), 7.71 (d, J = 2.5 Hz, 1H), 7.46 (d, J = 2.1 Hz, 1H), 7.33 (dd, J = 8.8, 2.5 Hz, 1H), 7.30 (dd, J = 8.5, 2.1 Hz, 1H), 6.98 (d, J = 8.3 Hz, 1H), 6.77 (d, J = 8.8 Hz, 1H), 3.22 (q, J = 6.8 Hz, 2H), 1.51 – 1.46 (m, 2H), 1.32 – 1.27 (m, 6H), 0.86 (t, J = 6.7 Hz, 3H); ^{13}C NMR (DMSO- d_6) δ 165.4, 151.8, 148.3, 144.4, 132.5, 129.8, 128.4, 126.8, 123.5, 120.3, 118.7, 118.6, 116.7, 31.0 (2C), 29.1, 26.2, 22.1, 13.9; HPLC purity 99.6%; HRMS (ESI+) calcd for C₁₉H₂₁Cl₂NO₃ 382.0971 (M + H⁺, $^{35}\text{Cl}_2$), found 382.0978.

1-[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl]methanamine (31): The title compound was prepared following a literature procedure.^[51] To a stirred suspension of LiAlH₄ (114 mg, 3.0 mmol) in dry Et₂O (20 ml) was added dropwise a solution of nitrile **26** (294 mg, 1.0 mmol) in dry Et₂O (3 ml) at 0 °C. Stirring was continued at the same temperature for further 0.5 h, then the cooling bath was removed and stirring continued at room temperature for 3 h. After re-cooling to 0 °C, the reaction mixture was quenched by dropwise addition of H₂O (0.5 ml) and NaOH (0.5 ml of a 1.0 M solution). Diethyl ether was added and the mixture filtered. The filtrate was washed with brine, dried over Na₂SO₄, and concentrated *in vacuo* to give a pale-yellow oil, which was purified by flash chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂-MeOH 5:1) to give the pure product as pale-yellow oil (242 mg, 81%).

¹H NMR (DMSO-*d*₆) δ 7.67 (d, *J* = 2.5 Hz, 1H), 7.29 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.20 (d, *J* = 1.7 Hz, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 6.94 (dd, *J* = 8.1, 1.8 Hz, 1H), 6.60 (d, *J* = 8.9 Hz, 1H), 3.74 (overlapping s, 3H), 3.73 (overlapping s, 2H), 3.17 (s, 2H); ¹³C NMR (DMSO-*d*₆) δ 152.6, 150.5, 142.9, 140.8, 129.6, 128.2, 126.0, 122.6, 121.0, 119.4, 117.2, 112.3, 55.7, 45.4; HPLC purity 95.1%.

N-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]-5-methyl-1,2-oxazole-3-carboxamide (32): The title compound was prepared following a literature procedure.^[50] The crude material was purified by flash chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂-MeOH 5:1) to give the pure product as a pale-yellow dense oil (107 mg, 32% on a 0.81 mmolar scale), which was used directly in the next step.

¹H NMR (DMSO-*d*₆) δ 9.27 (t, *J* = 6.3 Hz, 1H), 7.68 (d, *J* = 2.5 Hz, 1H), 7.28 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.17 (d, *J* = 1.8 Hz, 1H), 7.02 (d, *J* = 8.1 Hz, 1H), 6.92 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.64 (d, *J* = 8.9 Hz, 1H), 6.56 (d, *J* = 0.9 Hz, 1H), 4.44 (d, *J* = 6.2 Hz, 2H), 3.73 (s, 3H), 2.46 (d, *J* = 0.8 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 171.3, 158.9, 158.8, 152.3, 150.5, 141.6, 137.3, 129.7, 128.3, 126.3, 122.8, 121.1, 119.9, 117.6, 112.8, 101.4, 55.7, 42.0, 11.8; HPLC purity 94.4%.

N-[4-(2,4-Dichlorophenoxy)-3-hydroxybenzyl]-5-methyl-1,2-oxazole-3-carboxamide (33): The title compound was prepared following the General Procedure C with **32** as the starting material. The crude material was purified by preparative HPLC to give the pure product as a white solid (75 mg, 76% on a 0.25 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 9.76 (s, 1H), 9.25 (t, *J* = 6.2 Hz, 1H), 7.66 (d, *J* = 2.5 Hz, 1H), 7.28 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.97 – 6.95 (m, 2H), 6.78 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.63 (d, *J* = 8.9 Hz, 1H), 6.56 (d, *J* = 0.7 Hz, 1H), 4.37 (d, *J* = 6.2 Hz, 2H), 2.47 (br s, 3H); ¹³C NMR (DMSO-*d*₆) δ 171.3, 158.9, 158.7, 152.6, 148.7, 140.5, 137.2, 129.6, 128.2, 125.9, 122.7, 121.6, 118.7, 117.4, 116.2, 101.3, 41.7, 11.8; HPLC purity 99.7%; HRMS (ESI+) calcd for C₁₈H₁₅Cl₂N₂O₄ 393.0403 (M+H⁺, ³⁵Cl₂), found 393.0405.

3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzonitrile (34): The title compound was prepared following the same procedure as described for the synthesis of compound **26**. Purification by re-crystallization from MeOH provided the pure product as a pale-yellow solid (2.20 g, 75% on a 10 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 8.15 (d, *J* = 2.0 Hz, 1H), 7.69 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.34 (d, *J* = 2.3 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 7.10 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.76 (d, *J* = 8.6 Hz, 1H), 3.76 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 156.8, 151.7, 140.4, 134.2, 133.1, 131.0, 123.4, 122.3, 121.1, 117.6, 116.1, 114.2, 105.7, 56.4; HPLC purity 94.5%.

1-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)phenyl]methanamine (35): The title compound was prepared following the same procedure as described for the synthesis of compound **31**. The crude material was purified by flash chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂–MeOH 5:1) to give the pure product as a yellow oil (1.37 g, 86% on a 5.3 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 7.51 (d, *J* = 2.0 Hz, 1H), 7.25 (d, *J* = 2.4 Hz, 1H), 7.19 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.99 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 1H), 6.73 (d, *J* = 8.4 Hz, 1H), 3.79 (s, 3H), 3.67 (s, 2H), 3.17 (s, 2H); ¹³C NMR (DMSO-*d*₆) δ 151.2, 150.4, 143.1, 140.9, 128.8 (2C), 127.0, 122.4, 120.8, 120.6, 117.6, 113.7, 56.2, 44.5; HPLC purity 91.3%.

***N*-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-5-methyl-1,2-oxazole-3-carboxamide (36a)**, ***N*-[3-chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-5-phenyl-1,2-oxazole-3-carboxamide (36b)**, ***N*-[3-chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-2-(3-methyl-1,2-oxazol-5-yl)acetamide (36c)**, and ***N*-[3-chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-6-methoxynaphthalene-2-carboxamide (36d)** were prepared following a literature procedure^[50] with **35** and the following carboxylic acids as the starting materials: 5-methylisoxazole-3-carboxylic acid to give **36a** as a colorless dense oil (255 mg, 34% on a 1.84 mmolar scale), 5-phenylisoxazole-3-carboxylic acid to give **36b** as a pale-yellow oil (355 mg, 50% on a 1.50 mmolar scale), 2-(3-methylisoxazol-5-yl)acetic acid to give **36c** as a yellow dense oil (500 mg, 79% on a 1.50 mmolar scale), and 6-hydroxynaphthalene-2-carboxylic acid to give **36d** as an off-white solid (600 mg, 85% on a 1.50 mmolar scale).

***N*-[3-Chloro-4-(4-chloro-2-hydroxyphenoxy)benzyl]-5-methyl-1,2-oxazole-3-carboxamide (37a):** The title compound was prepared following the General Procedure C with **36a** as the starting material. Purification by preparative HPLC gave the pure product as a white solid (145 mg, 62% on a 0.59 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 10.19 (br s, 1H), 9.27 (t, *J* = 6.2 Hz, 1H), 7.46 (d, *J* = 2.0 Hz, 1H), 7.19 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.99 (d, *J* = 2.4 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 1H), 6.84 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.73 (d, *J* = 8.4 Hz, 1H), 6.54 (d, *J* = 0.9 Hz, 1H), 4.37 (d, *J* = 6.2 Hz, 2H), 2.46 (d, *J* = 0.7 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 171.3, 158.8 (2C), 151.6, 149.7, 141.8, 134.8, 129.3, 128.7, 127.5, 122.1, 122.0, 119.3, 117.3, 117.0, 101.3, 41.3, 11.8; HPLC purity 99.0%; HRMS (ESI+) calcd for C₁₈H₁₅Cl₂N₂O₄ 393.0403 (M+H⁺, ³⁵Cl₂), found 393.0406.

***N*-[3-Chloro-4-(4-chloro-2-hydroxyphenoxy)benzyl]-5-phenyl-1,2-oxazole-3-carboxamide (37b):** The title compound was prepared following the General Procedure C with **36b** as the starting material. Purification by preparative HPLC gave the pure product as a white solid (227 mg, 66% on a 0.76 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 10.21 (s, 1H), 9.41 (t, *J* = 6.0 Hz, 1H), 7.95 – 7.92 (m, 2H), 7.58 – 7.50 (m, 4H), 7.39 (s, 1H), 7.23 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.00 (d, *J* = 2.4 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 1H), 6.84 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.74 (d, *J* = 8.4 Hz, 1H), 4.42 (d, *J* = 6.0 Hz, 2H); ¹³C NMR (DMSO-*d*₆) δ 170.5, 159.5, 158.6, 151.6, 149.8, 141.8, 134.7, 130.9, 129.4, 129.3 (2C), 128.7, 127.6, 126.3, 125.8 (2C), 122.1, 122.0, 119.4, 117.3, 117.0, 99.9, 41.4; HPLC purity 99.4%; HRMS (ESI+) calcd for C₂₃H₁₇Cl₂N₂O₄ 455.0560 (M+H⁺, ³⁵Cl₂), found 455.0558.

***N*-[3-Chloro-4-(4-chloro-2-hydroxyphenoxy)benzyl]-2-(3-methyl-1,2-oxazol-5-yl)acetamide (37c):** The title compound was prepared following the General Procedure C

with **36c** as the starting material. Purification by preparative HPLC gave the pure product as pale-yellow solid (178 mg, 37% on a 1.18 mmolar scale).

^1H NMR (DMSO- d_6) δ 10.20 (s, 1H), 8.68 (t, J = 5.8 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.14 (dd, J = 8.5, 2.0 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 8.6 Hz, 1H), 6.85 (dd, J = 8.6, 2.4 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 6.19 (s, 1H), 4.24 (d, J = 5.8 Hz, 2H), 3.70 (s, 2H), 2.19 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 167.0, 166.5, 159.5, 151.6, 149.7, 141.8, 134.9, 129.1, 128.7, 127.4, 122.2, 122.0, 119.3, 117.3, 117.0, 103.8, 41.3, 33.6, 11.0; HPLC purity 98.6%; HRMS (ESI+) calcd for $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{N}_2\text{O}_4$ 407.0560 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$), found 407.0546.

N-[3-Chloro-4-(4-chloro-2-hydroxyphenoxy)benzyl]-6-hydroxynaphthalene-2-carboxamide (37d):

The title compound was prepared following the General Procedure C with **36c** as the starting material. Purification by preparative HPLC gave the pure product as a white solid (304 mg, 52% on a 1.28 mmolar scale).

^1H NMR (DMSO- d_6) δ 10.18 (s, 1H), 10.01 (s, 1H), 9.07 (t, J = 6.1 Hz, 1H), 8.36 (br s, 1H), 7.87 – 7.84 (m, 2H), 7.74 (d, J = 8.7 Hz, 1H), 7.50 (d, J = 2.0 Hz, 1H), 7.23 (dd, J = 8.5, 2.1 Hz, 1H), 7.16 – 7.13 (m, 2H), 6.99 (d, J = 2.3 Hz, 1H), 6.89 (d, J = 8.6 Hz, 1H), 6.84 (dd, J = 8.6, 2.4 Hz, 1H), 6.75 (d, J = 8.4 Hz, 1H), 4.46 (d, J = 5.8 Hz, 2H); ^{13}C NMR (DMSO- d_6) δ 166.4, 156.9, 151.4, 149.7, 141.9, 136.1, 135.8, 130.6, 129.2, 128.6, 128.3, 127.5, 127.4, 126.6, 126.0, 124.3, 122.1, 121.9, 119.4, 119.3, 117.4, 116.9, 108.6, 41.7; HPLC purity 97.7%; HRMS (ESI+) calcd for $\text{C}_{25}\text{H}_{17}\text{Cl}_2\text{NO}_4$ 454.0607 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$), found 454.0588.

N-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]aniline (38a): To a stirred solution of the benzyl bromide **6** (181 mg, 0.50 mmol) in dry THF (3 ml) was added potassium carbonate (207 mg, 1.5 mmol, 3.0 equiv) followed by aniline (1.5 mmol, 3.0 equiv). The mixture was refluxed for 16 h, cooled to room temperature, poured into water (30 ml), and extracted with diethyl ether (2 $\times$ 20 ml). The combined organic phases were washed with brine, dried over Na_2SO_4 , and concentrated *in vacuo* to give a yellow oil, which was purified by flash chromatography on silica gel (hexanes, then hexanes–EtOAc 3:1 to 3:2) to give the pure product as pale-yellow oil (90 mg, 48%).

^1H NMR (DMSO- d_6) δ 7.67 (d, J = 2.7 Hz, 1H), 7.29 (dd, J = 8.9, 2.6 Hz, 1H), 7.21 (d, J = 1.4 Hz, 1H), 7.09 – 6.96 (m, 4H), 6.64 – 6.59 (m, 3H), 6.52 (tt, J = 7.2, 1.0 Hz, 1H), 6.21 (t, J = 6.1 Hz, 1H), 4.26 (d, J = 6.1 Hz, 2H), 3.72 (s, 3H); ^{13}C NMR (DMSO- d_6) δ 152.4, 150.6, 148.6, 141.3, 138.8, 129.7, 128.8 (2C), 128.3, 126.2, 122.8, 121.0, 119.7, 117.5, 115.9, 112.5, 112.3 (2C), 55.7, 46.3; HPLC purity 95%; MS (ESI+) m/z 281 ($\text{M}+\text{H}^+ - \text{PhNH}_2$, $^{35}\text{Cl}_2$).

N-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]cyclohexanamine (38b): The title compound was prepared following the same procedure as described for the synthesis of compound **38a**. The crude yellow oil was purified by flash chromatography on silica gel (CH_2Cl_2 , then CH_2Cl_2 –MeOH 5:1) to give the pure product as a brown oil (213 mg, 54% on a 1.0 mmolar scale).

^1H NMR (DMSO- d_6) δ 7.68 (d, J = 2.5 Hz, 1H), 7.29 (dd, J = 8.9, 2.6 Hz, 1H), 7.19 (d, J = 1.6 Hz, 1H), 6.99 (d, J = 8.1 Hz, 1H), 6.94 (dd, J = 8.1, 1.6 Hz, 1H), 6.62 (d, J = 8.8 Hz, 1H), 3.74 (s, 2H), 3.73 (s, 3H), 2.40 (m, 1H), 1.88 – 1.84 (m, 2H), 1.70 – 1.66 (m, 3H), 1.54 (m, 1H), 1.25 – 1.02 (m, 5H); ^{13}C NMR (DMSO- d_6) δ 152.5, 150.5, 141.1, 129.7, 128.3 (2C), 126.1, 122.7, 120.9, 120.4, 117.4, 113.0, 55.7, 55.4, 49.6, 48.6, 32.8, 25.9, 24.4 (2C); HPLC purity 95.5%; MS (ESI+) m/z 380 ($\text{M}+\text{H}^+$, $^{35}\text{Cl}_2$).

N-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]hexan-1-amine (38c): The title compound was prepared following the same procedure as described for the synthesis of compound **38a**. The crude yellow oil was purified by flash chromatography on silica gel (CH₂Cl₂, then CH₂Cl₂–MeOH 5:1 to 4:1) to give the pure product as a pale-yellow oil (115 mg, 30% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 7.67 (d, *J* = 2.5 Hz, 1H), 7.29 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.18 (d, *J* = 1.5 Hz, 1H), 6.99 (d, *J* = 8.1 Hz, 1H), 6.93 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.62 (d, *J* = 8.9 Hz, 1H), 3.73 (s, 3H), 3.70 (s, 2H), 2.50 (m, 1H), 1.45 – 1.40 (m, 2H), 1.33 – 1.22 (m, 8H), 0.86 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 152.5, 150.5, 141.1, 139.5, 129.6, 128.2, 126.1, 122.7, 120.9, 120.4, 117.3, 113.0, 55.7, 52.6, 48.7, 31.3, 29.4, 26.5, 22.1, 13.9; HPLC purity 96.6%; MS (ESI+) *m/z* 382 (M+H⁺, ³⁵Cl₂).

2-(2,4-Dichlorophenoxy)-5-[(phenylamino)methyl]phenol (39a): The title compound was prepared following the General Procedure B with **38a** as the starting material. Purification by preparative HPLC gave the pure product as a yellow dense oil (33 mg, 38% on a 0.24 mmolar scale).

¹H NMR (DMSO) δ 9.76 (br s, 1H), 7.65 (d, *J* = 2.5 Hz, 1H), 7.29 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.05 (dd, *J* = 8.3, 7.4 Hz, 2H), 6.99 (d, *J* = 1.8 Hz, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 6.82 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.63 (d, *J* = 8.9 Hz, 1H), 6.57 (d, *J* = 7.7 Hz, 2H), 6.51 (t, *J* = 7.3 Hz, 1H), 6.21 (t, *J* = 6.0 Hz, 1H), 4.20 (d, *J* = 6.0 Hz, 2H); ¹³C NMR (DMSO) δ 152.6, 148.9, 148.6, 140.3, 138.6, 129.5, 128.8 (2C), 128.2, 125.8, 122.7, 121.4, 118.2, 117.4, 115.9, 115.7, 112.2 (2C), 46.0; HPLC purity 99.4%; MS (ESI+) *m/z* 360 (M+H⁺, ³⁵Cl₂); HRMS (ESI+) calcd for C₁₉H₁₆Cl₂NO₂ 360.0553 (M+H⁺, ³⁵Cl₂), found 360.0543.

5-[(Cyclohexylamino)methyl]-2-(2,4-dichlorophenoxy)phenol (39b): The title compound was prepared following the General Procedure B with **38b** as the starting material. Purification by preparative HPLC gave the pure product as an off-white solid (38 mg, 20% on a 0.52 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 9.63 (br s, 1H), 7.66 (d, *J* = 2.5 Hz, 1H), 7.29 (dd, *J* = 8.9, 2.6 Hz, 1H), 7.00 (d, *J* = 1.9 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.78 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.62 (d, *J* = 8.9 Hz, 1H), 3.65 (s, 2H), 2.50 (m, 1H), 2.37 (m, 1H), 1.85 – 1.82 (m, 2H), 1.70 – 1.65 (m, 2H), 1.54 (m, 1H), 1.23 – 0.99 (m, 5H); ¹³C NMR (DMSO-*d*₆) δ 152.7, 148.5, 140.0, 129.5, 128.1, 125.7, 122.6, 121.3 (2C), 119.0, 117.3, 116.7, 55.4, 49.5, 32.9 (2C), 25.9, 24.4 (2C); HPLC purity 100%; MS (ESI+) *m/z* 366 (M+H⁺, ³⁵Cl₂), HRMS (ESI+) calcd for C₁₉H₂₂Cl₂NO₂ 366.1022 (M+H⁺, ³⁵Cl₂), found 366.1006.

2-(2,4-Dichlorophenoxy)-5-[(hexylamino)methyl]phenol (39c): The title compound was prepared following the General Procedure C with **38c** as the starting material. Purification by preparative HPLC gave the pure product as an off-white solid (50 mg, 47% on a 0.29 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 9.71 (br s, 1H), 7.66 (d, *J* = 2.6 Hz, 1H), 7.29 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.97 (d, *J* = 1.8 Hz, 1H), 6.91 (d, *J* = 8.1 Hz, 1H), 6.77 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.62 (d, *J* = 8.9 Hz, 1H), 3.60 (s, 2H), 2.50 – 2.45 (t overlapping with DMSO, *J* = 7.1 Hz, 2H), 1.45 – 1.38 (m, 2H), 1.32 – 1.22 (m, 6H), 0.86 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 152.7, 148.6, 140.1, 139.5, 129.5, 128.1, 125.7, 122.6, 121.2, 119.1, 117.3, 116.7, 52.6, 48.8, 31.3, 29.5, 26.6, 22.1, 14.0; HPLC purity 100%; MS (ESI+) *m/z* 368 (M+H⁺, ³⁵Cl₂); HRMS (ESI+) calcd for C₁₉H₂₄Cl₂NO₂ 368.1179 (M+H⁺, ³⁵Cl₂), found 368.1182.

N-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]hexan-1-amine (40a): The title compound was prepared following a literature procedure.^[52] The product was obtained as a pale yellow oil in a yield of 165 mg (43% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 7.48 (d, *J* = 1.9 Hz, 1H), 7.25 (d, *J* = 2.3 Hz, 1H), 7.18 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.00 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.72 (d, *J* = 8.3 Hz, 1H), 3.79 (s, 3H), 3.62 (s, 2H), 2.43 (t, *J* = 7.0 Hz, 2H), 1.43 – 1.36 (m, 2H), 1.31 – 1.20 (m, 7H), 0.85 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 151.3, 150.7, 143.0, 137.7, 129.6, 128.9, 127.8, 122.2, 121.0, 120.7, 117.4, 113.8, 56.2, 51.8, 48.6, 31.3, 29.5, 26.5, 22.1, 13.9; HPLC purity 99.1%; MS (ESI+) *m/z* 369 (M+H⁺, ³⁵Cl₂).

N-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]aniline (40b): The title compound was prepared following a literature procedure.^[52] The product was obtained as pale-yellow oil in a yield of 315 mg (85% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 7.50 (d, *J* = 2.0 Hz, 1H), 7.25 (overlapping d, *J* = 2.4 Hz, 1H), 7.23 (overlapping dd, *J* = 8.3, 2.0 Hz, 1H), 7.04 (dd, *J* = 8.5, 7.3 Hz, 2H), 7.00 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 6.73 (d, *J* = 8.3 Hz, 1H), 6.56 (dd, *J* = 8.5, 0.8 Hz, 2H), 6.52 (fine splitting tt, *J* = 8.2, 1.0 Hz, 1H), 6.24 (t, *J* = 6.1 Hz, 1H), 4.22 (d, *J* = 6.2 Hz, 2H), 3.78 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 151.4, 151.0, 148.3, 142.7, 136.6, 129.1, 128.8 (3C), 127.2, 122.3, 121.3, 120.7, 117.4, 115.9, 113.8, 112.3 (2C), 56.2, 45.3; HPLC purity 98.5%; MS (ESI+) *m/z* 361 (M+H⁺, ³⁵Cl₂).

5-Chloro-2-[2-chloro-4-[(hexylamino)methyl]phenoxy]phenol (41a): The title compound was prepared following the General Procedure C with **40a** as the starting material. Purification by preparative HPLC gave the pure product as a white crystalline solid (110 mg, 55% on a 0.43 mmolar scale), which was estimated by elemental analysis to be a TFA salt with the formula C₁₉H₂₃Cl₂NO₂ • 0.85TFA • 0.4H₂O.

¹H NMR (DMSO-*d*₆) δ 10.31 (br s, 1H), 8.60 (br s, 2H), 7.69 (d, *J* = 2.2 Hz, 1H), 7.33 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.03 (d, *J* = 2.5 Hz, 1H), 7.00 (d, *J* = 8.6 Hz, 1H), 6.90 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.77 (d, *J* = 8.4 Hz, 1H), 4.09 (s, 2H), 2.89 (t, *J* = 7.6 Hz, 2H), 1.62 – 1.54 (m, 2H), 1.34 – 1.24 (m, 6H), 0.87 (t, *J* = 6.8 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 151.7, 150.1, 141.8, 130.1, 128.7 (2C), 128.4, 122.2 (2C), 119.1, 117.0 (2C), 51.1, 48.1, 31.1, 28.4, 26.3, 22.1, 13.9; HPLC purity 99.3%; HRMS (ESI+) calcd for C₁₉H₂₄Cl₂NO₂ 368.1179 (M+H⁺, ³⁵Cl₂), found 368.1162.

5-Chloro-2-[2-chloro-4-[(phenylamino)methyl]phenoxy]phenol (41b): The title compound was prepared following the General Procedure C with **40b** as the starting material. Purification by preparative HPLC gave the pure product as a colorless oil, which was a TFA salt. The material was subsequently dissolved in EtOAc (25 ml) and treated with solid NaHCO₃. After stirring for 1 h at room temperature, the suspension was filtered and the solution concentrated *in vacuo* to give a pale-yellow dense oil (204 mg, 74% on a 0.77 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 10.23 (br s, 1H), 7.49 (d, *J* = 2.0 Hz, 1H), 7.23 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.04 (dd, *J* = 8.5, 7.3 Hz, 2H), 6.99 (d, *J* = 2.4 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 1H), 6.84 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.71 (d, *J* = 8.3 Hz, 1H), 6.56 (dd, *J* = 8.5, 0.9 Hz, 2H), 6.52 (fine splitting tt, *J* = 7.3, 1.0 Hz, 1H), 6.24 (t, *J* = 6.1 Hz, 1H), 4.21 (d, *J* = 6.1 Hz, 2H); ¹³C NMR (DMSO-*d*₆) δ 151.3, 149.9, 148.3, 141.8, 136.3, 128.9 (2C), 128.8, 128.7, 127.1, 122.2, 122.0, 119.2, 117.2, 117.0, 115.9, 112.3 (2C), 45.3; HPLC purity 97.5%; HRMS (ESI+) calcd for C₁₉H₁₆Cl₂NO₂ 360.0553 (M+H⁺, ³⁵Cl₂), found 360.0557.

1-[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl]propan-1-one (42a): The title compound was prepared following a literature procedure.^[53] The crude material was purified by flash chromatography on silica gel (hexanes, then hexanes–EtOAc 3:1 to 3:2) to provide the pure product as pale-red oil (259 mg, 80% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 7.75 (d, *J* = 2.2 Hz, 1H), 7.65 (d, *J* = 1.9 Hz, 1H), 7.61 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.38 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 1H), 3.85 (s, 3H), 3.05 (q, *J* = 7.2 Hz, 2H), 1.09 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 199.2, 150.8, 150.0, 147.7, 133.8, 130.0, 128.7, 128.0, 124.4, 121.8, 120.1, 118.9, 112.1, 55.9, 31.1, 8.2; HPLC purity 89.9%; MS (ESI+) *m/z* 325 (M+H⁺, ³⁵Cl₂).

[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl](phenyl)methanone (42b): The title compound was prepared following a literature procedure.^[53] The crude material was purified by flash chromatography on silica gel (hexanes, then hexanes–EtOAc 3:1 to 3:2) to provide the pure product as a pale-yellow oil (254 mg, 68% on a 1.0 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 7.77 – 7.75 (m, 3H), 7.68 (m, 1H), 7.59 – 7.53 (m, 3H), 7.40 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.29 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.03 (d, *J* = 3.1 Hz, 1H), 7.01 (d, *J* = 3.6 Hz, 1H), 3.85 (s, 3H); ¹³C NMR (DMSO-*d*₆) δ 194.5, 150.8, 150.0, 147.6, 137.1, 133.8, 132.6, 130.1, 129.5 (2C), 128.7, 128.6 (2C), 128.1, 124.5, 124.0, 120.4, 118.5, 113.8, 56.0; HPLC purity 94.4%; MS (ESI+) *m/z* 373 (M+H⁺, ³⁵Cl₂).

1-[4-(2,4-Dichlorophenoxy)-3-hydroxyphenyl]propan-1-one (43a): The title compound was prepared following the General Procedure B with **42a** as the starting material. Purification by preparative HPLC gave the pure product as a white solid (25 mg, 11% on a 0.74 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 10.11 (s, 1H), 7.74 (d, *J* = 2.5 Hz, 1H), 7.54 (d, *J* = 2.0 Hz, 1H), 7.47 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.37 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.98 (d, *J* = 8.3 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 2.98 (q, *J* = 7.1 Hz, 2H), 1.07 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (DMSO-*d*₆) δ 199.1, 151.2, 148.3, 146.5, 133.9, 129.9, 128.5, 127.4, 124.0, 120.3, 119.8, 119.6, 116.2, 31.0, 8.2; HPLC purity 99.4%; HRMS (ESI–) calcd for C₁₄H₁₁Cl₂O₃ 309.0091 (M–H⁺), found 309.0091.

[4-(2,4-Dichlorophenoxy)-3-hydroxyphenyl](phenyl)methanone (43b): The title compound was prepared following the General Procedure B with **42b** as the starting material. Purification by preparative HPLC gave the pure product as a pale-yellow oil (30 mg, 13% on a 0.64 mmolar scale).

¹H NMR (DMSO-*d*₆) δ 10.21 (s, 1H), 7.75 – 7.72 (m, 3H), 7.67 (m, 1H), 7.58 – 7.55 (m, 2H), 7.70 (overlapping d, *J* = 1.9 Hz, 1H), 7.38 (overlapping dd, *J* = 8.8, 2.5 Hz, 1H), 7.20 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.03 (d, *J* = 8.3 Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 1H); ¹³C NMR (DMSO-*d*₆) δ 194.7, 151.2, 148.2, 146.4, 137.3, 133.9, 132.4, 129.9, 129.4 (2C), 128.6, 128.5 (2C), 127.5, 124.1, 122.1, 119.8, 119.7, 118.3; HPLC purity 99.5%; MS (ESI+) *m/z* 359 (M+H⁺, ³⁵Cl₂); HRMS (ESI+) calcd for C₁₉H₁₃Cl₂O₃ 359.0236 (M+H⁺, ³⁵Cl₂), found 359.0229.

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Abbreviations

acetyl-CoA	acetyl coenzyme A
ACCase	acetyl-CoA-carboxylase
ACP	acyl carrier protein
ADMET	absorption, distribution, metabolism, excretion, toxicity
Caco-2	permeability through Caco-2 monolayer
CoA	coenzyme A
ENR	enoyl-ACP reductase
F (> 70)	probability of bioavailability more than 70%
FASII	type II fatty acid synthesis pathway
HFF	human foreskin fibroblasts
NADH	nicotinamide adenine dinucleotide
<i>P. falciparum</i>	<i>Plasmodium falciparum</i>
<i>T. gondii</i>	<i>Toxoplasma gondii</i>
TgENR	<i>Toxoplasma gondii</i> enoyl-ACP reductase
TPSA	topological polar surface area
Sw	solubility in water

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Figure 1.

The crystal structure of the *Tg*ENR/NAD⁺/triclosan complex (A) with docked compound **16c** (B) and **37c** (C). The key residues which line the inhibitor binding pocket are shown in stick format along with the NAD⁺ co-factor, as well as triclosan (A) and compounds **16c** (B) and **37c** (C). The stick representations are colored red for oxygen, blue for nitrogen, orange for phosphorus, magenta for chlorine and green (**16c**, **37c** and triclosan) or yellow (*Tg*ENR) for carbon. The figure was produced using PyMOL.

Figure 2. Xenogen camera images of mice showing the reduced effect of compounds **16c** and **37c** on parasite burden compared to triclosan at a concentration of 10mg/ml.

Figure 3. Comparison of 10mg/kg of triclosan and compounds **16c** and **37c**. Compounds **16c** and **37c** had no effect on *T. gondii* *in vivo* at 10mg/kg at which triclosan demonstrated efficacy ($p > 0.05$).

Figure 4.

Compounds **16c** and **37c** are effective in reducing parasite burden at 75mg/kg on the 5th day after infection. This experiment was replicated twice. Although, at 75 mg/kg **16c** and **37c** reduced parasite growth *in vivo* at day 5 post infection ($p < 0.055$ and $p < 0.0079$ on days 4 and 5 by Mann Whitney), this effect was not evident in mice treated with 50mg/kg (results not shown).

Scheme 1.

Synthesis of 5- and 4'-triazole analogs of triclosan. *Reagents and conditions:* (a) neopentyl glycol, H₃NSO₃, PhMe, 110 °C, 3 h, 87%; (b) 1. For **3**, 1,3-dichloro-4-fluorobenzene, Cs₂CO₃, DMF, 130 °C, 14 h, 51%; 2. for **11**, 3-chloro-4-fluorobenzaldehyde, Cs₂CO₃, DMF, 125 °C, 16 h, 92%; (c) PPTS, wet acetone, reflux, 2.5 h, 100%; (d) NaBH₄, MeOH, RT, 1.5 h. For **5**, 84%; for **12**, 100%; (e) CBr₄, PPh₃, THF, RT, 1. For **6**, 14 h, 83%; 2. for **13**, 2 h, 80%; (f) NaN₃, DMF, 1. For **7**, 110 °C, 7 h, 71%; 2. for **14**, 100 °C, 14 h, 56%; (g) sodium ascorbate, CuSO₄·5H₂O, 1-alkyne, *t*-BuOH-H₂O (1:1), RT, 14–21 h. For **8a**, R = *n*-Bu, 86%; for **8b**, R = Ph, 100%; for **8c**, R = TMS, 100%; for **8d**, R = -(CH₂)₂OH, 81%; for **15a**, R = *n*-Bu, 80%; for **15b**, R = Ph, 80%; for **15c**, R = TMS, 82%; (h) 1. For **8c**, R = TMS, *n*-Bu₄NF, THF, RT, 23 h, 57%; 2. Bu₄NI, CH₂Cl₂, RT, 5 min, then -78 °C, BCl₃, 15 min, then RT, 1.5 h. For **9a**, R = *n*-Bu, 43%; for **9b**, R = Ph, 26%; for **9c**, R = H, 33%; (i) 1. For **15c**, R = TMS, *n*-Bu₄NF, THF, RT, 20 h, 76%; 2. BBr₃, CH₂Cl₂, -78 °C to RT within 1 h, then RT, 3 h. For **16a**, R = *n*-Bu, 48%; for **16b**, R = Ph, 63%; for **16c**, R = H, 65%.

Scheme 2.

Synthesis of 5- and 4'-isoxazole analogs of triclosan. *Reagents and conditions:* (a) liquid H₂O-EtOH-ice (1:1:2), H₂NOH·HCl, 50% aq NaOH, RT, 75 min, 90%; (b) NCS, DMF, RT, 1.5 h, 100%; (c) sodium ascorbate, CuSO₄·5H₂O, KHCO₃, 1-alkyne, *t*-BuOH-H₂O (1:1), RT, 1.5 – 2.5 h. For **19a**, R = *n*-Bu, 81%; for **19b**, R = Ph, 98%; for **19c**, R = TMS, 95%; for **23a**, R = *n*-Bu, 70%; for **23b**, R = Ph, 88%; (d) Bu₄NI, CH₂Cl₂, RT, 5 min, then BCl₃, –78 °C, 15 min, then RT, 1.5 – 2.5 h. For **20a**, R = *n*-Bu, 36%; for **20b**, R = Ph, 30%; for **20c**, R = TMS, 52%; for **24a**, R = *n*-Bu, 27%; for **24b**, R = Ph, 33%.

Scheme 3.

Substitution of triclosan at positions 5 and 4' with cyano, carbamoyl, acyl, aminomethyl, and (acylamino)methyl groups. *Reagents and conditions:* (a) 1. 2,4-dichlorophenol, Cs₂CO₃, DMF, 120 °C, 21 h, 65% for **26**; 2. 3-chloro-4-fluorobenzonitrile, Cs₂CO₃, DMF, 100 °C, 16 h, 75% for **34**; (b) Bu₄NI, CH₂Cl₂, RT, 5 min, then BCl₃, -78 °C, 15 min, then RT, 2 h. For **27**, 82%; for **30a**, R = H, R¹ = Ph, 51%; for **30b**, R = R¹ = *n*-Bu, 70%; for **30c**, RR¹ = -(CH₂)₂-O-(CH₂)₂-, 51%; for **39a**, R³ = Ph, 38%; for **39b**, R³ = *c*-Hex, 20%; for **43a**, R⁵ = Et, 11%; for **43b**, R⁵ = Ph, 13%; (c) 25% aq NaOH, EtOH, reflux, 21 h, 67%; (d) 1. carboxylic acid, DIPEA, RT, 10 min; 2. BOP-Cl, NHR¹R², DIPEA, RT; 3. 24 h; for **29a**, R = H, R¹ = Ph, 81%; for **29b**, R = R¹ = *n*-Bu, 100%; for **29c**, RR¹ = -(CH₂)₂-O-(CH₂)₂-, 77%; for **29d**, R = H, R¹ = *n*-Hex, 42%; or 3. 20 h; for **32**, 32%; or 3. 2 – 4.5 h; for **36a**, 34%; for **36b**, 50%; for **36c**, 79%; for **36d**, 85%; (e) 1. LiAlH₄, Et₂O, 0 °C, 0.5 h then RT, 3 h; 2. H₂O, 1.0 M NaOH. For **31**, 81%; for **35**, 95%; (f) BBr₃, CH₂Cl₂, -78 °C to RT within 1 h, then RT, 3 h. For **30d**, R = H, R¹ = *n*-Hex, 69%; for **33**, 76%; for **37a**, 62%; for **37b**, 66%; for **37c**, 37%; for **37d**, 52%; for **39c**, R³ = *n*-Hex, 47%; for **41a**, R⁴ = *n*-Hex, 55%; for **41b**, R⁴ = Ph, 74%; (g) RNH₂, K₂CO₃, reflux, 24 h. For **38a**, R³ = Ph, 48%; for **38b**, R³ = *c*-Hex, 54%; for **38c**, R³ = *n*-Hex, 30%; (h) 1. RNH₂, powdered molecular sieves 4 Å, CH₂Cl₂, RT, 6 – 8 h; 2. NaBH(OAc)₃, RT, 24 h. For **40a**, R⁴ = *n*-Hex, 43%; for **40b**, R⁴ = Ph, 85%; (i) 1. R⁵MgBr, 0 °C, 0.5 h, then RT, 18 h; 2. 1 M HCl. For **42a**, R⁵ = Et, 80%; for **42b**, R⁵ = Ph, 68%.

Table 1
Activity data and ADMET predictions for new series of triclosan-based *Tg*ENR inhibitors

Compound	Parasite tissue culture assay			<i>Tg</i> ENR enzyme assay (5 nM enzyme concentration)			Caco-2 Perm. $[h] \times 10^{-6}$ [cm/s]
	MIC ₅₀ [μ M] ^[a]	HFF ^a [μ M]	Cpd conc. [1 μ M]/% Inh. ^[b]	IC ₅₀ [nM]	95% Conf. Interval [nM] ^[c]	Cllog P ^[d]	
Triclosan	3	> 10	1/97	17	13–22	5.5	184

8d	> 10	> 10	1/12	nd		3.1	235

9a	> 10	> 10	1/94	38	30–48	5.5	214

9b	> 10	> 10	1/93	54	43–68	5.7	164

9c	10	> 10	1/93	130	87–206	3.6	233

16a	0.25	> 10	1/90	43	35–54	5.8	206

Compound	Parasite tissue culture assay		TgENR enzyme assay (5 nM enzyme concentration)			Clog <i>P</i> [<i>d</i>]	TPSA[<i>e</i>] [Å ²]	S _w [<i>f</i>] × 10 ⁻³ [mg/ml]	P[<i>g</i>] (>70)	Caco-2 Perm. [<i>h</i>] × 10 ⁻⁶ [cm/s]
	MIC ₅₀ [μM][<i>a</i>]	HFF ^a [μM]	Cpd conc. [1 μM]/ % Inh. [<i>b</i>]	IC ₅₀ [nM]	95% Conf. Interval [nM][<i>c</i>]					
16b	1	>10	1/82	nd		6.0	60.2	0.8	0.04	152
16c	0.25	>10	1/91	26	23–41	3.9	60.2	10	0.19	235
20a	>10	>10	1/59	nd		6.6	55.5	0.3	0.04	80
20b	>10	>10	1/40	nd		6.9	55.5	0.1	0.21	68
20c	7	>10	1/25	nd		7.4	55.5	0.7	0.21	69
24a	8	>10	1/92	18	14–24	6.8	55.5	0.3	0.04	67

Compound	Parasite tissue culture assay		TgENR enzyme assay (5 nM enzyme concentration)			Clog P[d]	TPSA[e] [Å ²]	S _w [f] × 10 ⁻³ [mg/ml]	R _f [g] (>70)	Caco-2 Perm. [h] × 10 ⁻⁶ [cm/s]
	MIC ₅₀ [μM][a]	HFF ^a [μM]	Cpd.conc. [1 μM]/ % Inh. [b]	IC ₅₀ [nM]	95% Conf. Interval [nM][c]
24b	10	> 10	1/88	28	22–36	7.0	55.5	0.1	0.21	65
27	2	> 10	1/97	24	16–36	4.6	53.2	1.1	0.78	233
30a	> 10	> 10	1/7	nd		5.4	58.6	0.1	0.20	118
30b	> 10	> 10	1/44	nd		6.7	49.8	1.0	0.04	183
30c	> 10	> 10	1/43	nd		3.7	59.0	28	0.06	235

Compound	Parasite tissue culture assay		TgENR enzyme assay (5 nM enzyme concentration)			Clog P[d]	TPSA[e] [Å ²]	S _w [f] × 10 ⁻³ [mg/ml]	P[g] (>70)	Caco-2 Perm. [h] × 10 ⁻⁶ [cm/s]
	MIC ₅₀ [μM][a]	HFF ^a [μM]	Cpd.conc. [1 μM]/ % Inh. [b]	IC ₅₀ [nM]	95% Conf. Interval [nM][c]					
30d	> 10	> 10	1/30	nd		6.2	58.6	0.4	0.21	61
33	10	nd	1/96	19	17-21	4.3	84.6	4.9	0.21	215
37a	5	nd	1/88	100	79-126	4.6	84.6	3.9	0.21	210
37b	> 10	nd	1/80	nd		6.4	84.6	0.2	0.21	99
37c	> 10	nd	1/96	33	27-40	3.6	84.6	4.1	0.29	201
37d	> 10	nd	1/89	nd		6.3	78.8	0.1	0.04	42

Compound	Parasite tissue culture assay		TgENR enzyme assay (5 nM enzyme concentration)			Clog P[d]	TPSA[e] [Å ²]	Swiff] × 10 ⁻³ [mg/ml]	R ² g] (>70)	Caco-2 Perm. [h] × 10 ⁻⁶ [cm/s]
	MIC ₅₀ [μM][a]	HFF ^a [μM]	Cpd.conc. [1 μM]/ % Inh. [b]	IC ₅₀ [nM]	95% Conf. Interval [nM][c]					
39a	10	>10	1/96	130	98–174	5.5	41.5	0.3	0.04	48
39b	4	>10	1/34	nd		5.9	41.5	0.7	0.04	68
39c	3	10–1	1/16	nd		6.5	41.5	0.7	0.04	9
41a	1	>10	1/25	nd		6.8	41.5	0.4	0.04	6
41b	>10	>10	1/94	31	26–37	5.8	41.5	0.2	0.04	37
43a	>10	>10	1/85	380	300–481	5.0	46.5	1.3	0.21	233

Compound	Parasite tissue culture assay		TgENR enzyme assay (5 nM enzyme concentration)			Clog P ^[d]	TPSA ^[e] [Å ²]	S _w [f] × 10 ⁻³ [mg/ml]	P ^[g] (>70)	Caco-2 Perm. [h] × 10 ⁻⁶ [cm/s]
	MIC ₅₀ [μM] ^[a]	HFF ^a [μM]	Cpd.conc. [1 μM]/ % Inh. ^[b]	IC ₅₀ [nM]	95% Conf. Interval [nM] ^[c]					
<div>43b</div> <div> </div>	> 10	>10	1/78	750	487–1150	6.1	46.5	0.2	0.20	136

^[a]HFF=human foreskin fibroblasts; Please see methods for details of assay. Herein, MIC₅₀ value refers to concentration where there is half the RFU in the compound treated culture relative to the DMSO control with subtraction of RFU for control fibroblasts without challenge. Data for the compounds that were best in the parasite assay are highlighted in turquoise blue; the next best are highlighted in green. In addition, data from two to four separate experiments performed at different times using the compounds that fell in the >1<10 μM and two in the >0.1<1 μM were tested. Concentrations utilized were 1.25, 2.50, 5.00, 10.0 μM for the compounds with activity >1<10 μM, and 0.100, 0.250, 0.5, 0.75 μM for the other two with activity >0.1<1 μM. For the enzyme data, those that had the highest activity are highlighted in turquoise blue. In the first column for ADMET, those that were selected to test in the murine model are highlighted in grey.

^[b]Cpd. Conc. [1 μM]/% Inh.= percentage of inhibition at compound concentration 1 μM.

^[c]95% confidence interval value.

^[d]Calculated using ChemDraw Ultra 7.0.

^[e]TPSA = topological polar surface area.

^[f]S_w = solubility in water.

^[g]F_h=probability of bioavailability more than 70%.

^[h]Caco-2 Perm.= permeability through Caco-2 monolayer.

Table 2

Activity data for triclosan-based inhibitors of *P. falciparum* ENR

Compound	SYBR Green D6 ^[a] IC ₅₀ [μM]	SYBR Green D6 IC ₅₀ [ng/ml]	SYBR D6 R ²	SYBR Green C235 ^[b] IC ₅₀ [μM]	SYBR Green C235 IC ₅₀ [ng/ml]	SYBR C235 R ²
9c	4.71	1583	0.87	nd ^[c]	>2000	N/A ^[d]
30a	nd	>2000	N/A	nd	>2000	N/A
30b	0.40	165.9	0.97	0.60	247	0.95
30c	nd	>2000	N/A	nd	>2000	N/A
30d	nd	>2000	N/A	nd	>2000	N/A
39a	4.56	1641	0.86	nd	>2000	N/A
39b	0.03	11.86	1.00	0.08	27.6	0.97
39c	2.75	856.5	0.93	nd	>2000	N/A
43a	2.75	856.5	0.93	nd	>2000	N/A
43b	nd	>2000	N/A	nd	>2000	N/A

^[a]CDC/Sierra Leone strain of *P. falciparum*.

^[b]TM91C235 (WRAIR, Thailand) strain of *P. falciparum*.

^[c]nd=not determined.

^[d]N/A=not active.