ChemPubSoc Europe

DOI: 10.1002/cmdc.201300050

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

Jozef Stec,[a, h] Alina Fomovska,[b, i] Gustavo A. Afanador,[c] Stephen P. Muench,[d] Ying Zhou,[b] Bo-Shiun Lai,[b] Kamal El Bissati,[b] Mark R. Hickman,[e] Patty J. Lee,[e] Susan E. Leed,[e] Jennifer M. Auschwitz,[e] Caroline Sommervile,[f] Stuart Woods,[f] Craig W. Roberts,[f] David Rice,[g] Sean T. Prigge,[c] Rima McLeod,*[b] and Alan P. Kozikowski*[a]

Through our focused effort to discover new and effective agents against toxoplasmosis, a structure-based drug design approach was used 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 analogues. 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 IC_{50} values of 250 nM against *Toxoplasma gondii* tachyzoites without apparent toxicity to the host cells. Their IC_{50} values against recombinant *Tg*ENR were found to be 43 and 26 nM, respectively. Additionally, 11 other analogues in this series had IC_{50} values ranging from 17 to 130 nM in the enzyme-based assay. 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 excellent starting points for the development of new medicines to effectively treat *Toxoplasma gondii* infections.

Introduction

Toxoplasma gondii is an apicomplexan protozoan responsible for toxoplasmosis, an infectious disease of warm-blooded animals, including humans.[1] *T. 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 the *T. gondii* life cycle, which includes 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 de-

[a] *Dr. J. Stec,[+] Prof. Dr. A. P. Kozikowski*
Drug Discovery Program
Department of Medicinal Chemistry and Pharmacognosy
University of Illinois at Chicago
833 South Wood Street, Chicago, IL 60612 (USA)
E-mail: kozikowa@uic.edu

[b] *A. Fomovska,[+] Dr. Y. Zhou,[+] B.-S. Lai, Prof. Dr. K. El Bissati, Dr. R. McLeod*
Department of Ophthalmology and Visual Sciences, Pediatrics (Infectious Diseases), Committees on Genetics, Immunology, and Molecular Medicine, Institute of Genomics and Systems Biology, and The College
The University of Chicago, Chicago, IL 60637 (USA)
E-mail: rmcleod@uchicago.edu

[c] *G. A. Afanador,[+] Dr. S. T. Prigge*
Johns Hopkins School of Public Health, Room E5132
615 North Wolfe Street, Baltimore MD 21205 (USA)

[d] *Prof. Dr. S. P. Muench[+]*
School of Biomedical Sciences, University of Leeds, Leeds, LS2 9JT (UK)

[e] *Dr. M. R. Hickman, P. J. Lee, S. E. Leed, J. M. Auschwitz*
Department of Discovery, Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Room 2N61, 503 Robert Grant Avenue, Silver Spring, MD 20910 (USA)

[f] *Dr. C. Sommervile, Dr. S. Woods, Prof. Dr. C. W. Roberts*
Strathclyde Institute of Pharmacy and Biomedical Sciences
University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE (UK)

[g] *Prof. Dr. D. Rice*
Department of Molecular Biology and Biotechnology
The University of Sheffield, Sheffield (UK)

[h] *Dr. J. Stec[+]*
Chicago State University, College of Pharmacy
DH 203-3, 9501 South King Drive, Chicago, IL 60628 (USA)

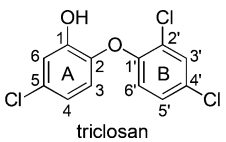
[i] *A. Fomovska[+]*
Mount Sinai School of Medicine
1 Gustave L. Levy Place, New York, NY 10029 (USA)

[+] *These authors contributed equally to this work.*

crease 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 an apicoplast.[9,10] A small circular genome and biochemical pathways such as isoprenoid and type II fatty acid biosynthesis systems were detected within this particular organelle.[11,12] The mechanism of the apicoplast-localized type II fatty acid biosynthesis pathway (FAS II) was initially studied in *Plasmodium 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 monofunctional enzymes known collectively as FAS II.[13,14] In contrast, the eukaryotic type I fatty acid biosynthesis system (FAS I) operates as a single multifunctional enzyme that catalyzes all the steps of the pathway. Acetyl-CoA carboxylase (ACCase), an enzyme responsible for the synthesis of malonyl-CoA, also 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. Because 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 antibacterial drugs that target FabI could act against protozoan parasites. Indeed, triclosan and other known FAS II inhibitors have been demonstrated to effectively target the apicoplast-associated fatty acid biosynthesis pathway.[23–26]



triclosan

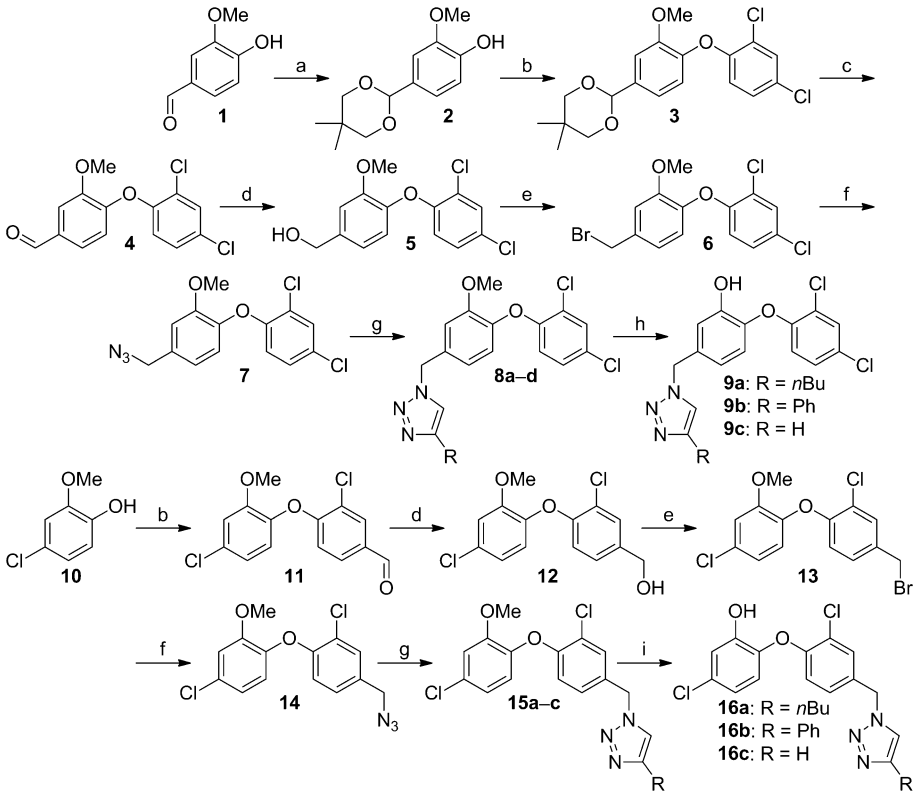
Triclosan belongs to the 2-hydroxydiphenyl ether family, and is widely used in household goods such as toothpastes, soaps, and plastics due to its activity against a broad spectrum of bacteria. It was initially suspected that this compound acts on bacterial cell membranes, but more recently it was revealed to inhibit bacterial lipid biosynthesis 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 nanomolar range for enzyme activity and low micromolar range toward 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 analogues.[31–34] Although some of the derivatives were equally or even more active than the 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 octa-arginine moiety 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] along with 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 enzyme activity in *T. gondii*, and in some cases *P. falciparum*. Computer-aided absorption, distribution, metabolism, excretion, toxicity (ADMET) predictions were used in the design of compounds with improved drug-like properties. We were particularly interested in improving the 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 insight into the discovery of new and effective inhibitors of *Tg*ENR.

Results and Discussion

Design and synthesis of the A ring triclosan derivatives

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 into its acetal **2** by following a published 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 *para*-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 bromide **6** with sodium azide provided the desired precursor **7** for the copper-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**.



Scheme 1. Synthesis of 5- and 4′-triazole analogues of triclosan. *Reagents and conditions*: a) neopentyl glycol, H_3NSO_3, PhMe, 110 °C, 3 h, 87%; b) 1. for **3**: 1,3-dichloro-4-fluorobenzene, Cs_2CO_3, DMF, 130 °C, 14 h, 51%, 2. for **11**: 3-chloro-4-fluorobenzaldehyde, Cs_2CO_3, DMF, 125 °C, 16 h, 92%; c) PPTS, wet acetone, reflux, 2.5 h, 100%; d) $NaBH_4$, MeOH, RT, 1.5 h (**5**: 84%, **12**: 100%); e) CBr_4, PPh_3, THF, RT, 1. for **6**: 14 h, 83%, 2. for **13**: 2 h, 80%; f) NaN_3, DMF, 1. for **7**: 110 °C, 7 h, 71%, 2. for **14**: 100 °C, 14 h, 56%; g) sodium ascorbate, $CuSO_4 \cdot 5H_2O$, 1-alkyne, *t*BuOH/H_2O (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 = $-(CH_2)_2OH$, 81%, for **15a**: R = *n*Bu, 80%, for **15b**: R = Ph, 80%, for **15c**: R = TMS, 82%; h) 1. for **8c**: R = TMS, nBu_4NF, THF, RT, 23 h, 57%, 2. Bu_4NI, CH_2Cl_2, RT, 5 min, then −78 °C, BCl_3, 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, nBu_4NF, THF, RT, 20 h, 76%, 2. BBr_3, CH_2Cl_2, −78 °C→RT within 1 h, then RT, 3 h, for **16a**: R = *n*Bu, 48%, for **16b**: R = Ph, 63%, for **16c**: R = H, 65%.

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

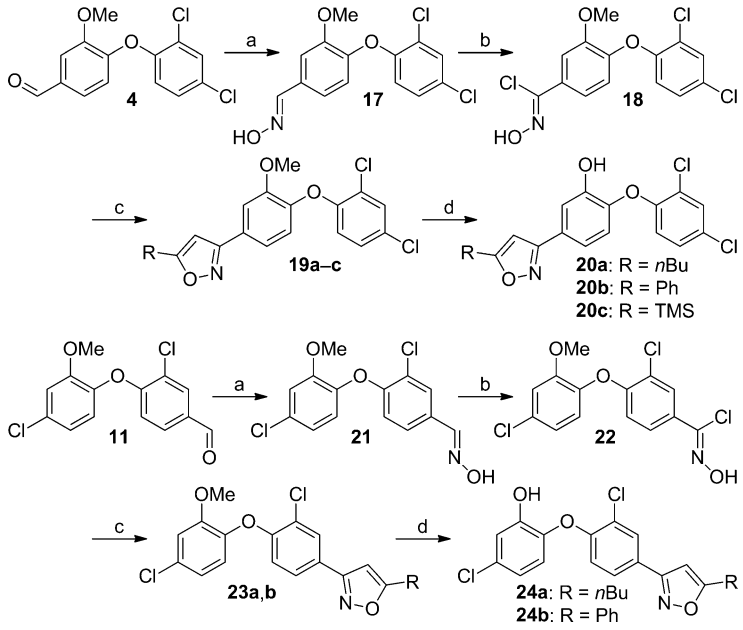
Triclosan derivatives bearing isoxazole groups at positions 5 and 4′ were also synthesized (Scheme 2). Intermediates **19a–c** and **23a,b** were prepared by following the Sharpless reference cited above.[45] Aldehydes **4** and **11** were converted in high yields into oximes **17** and **21**, respectively. Reaction of these oximes with *N*-chlorosuccinimide (NCS) gave the corresponding imidoyl chlorides **18** and **22**, which were immediately treated with the appropriate 1-alkynes to provide isoxazoles **19a–c** and **23a,b**, respectively. Tetrabutylammonium iodide/boron-trichloride-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 boron-tribromide-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 analogues of triclosan, **30a–d**. Reduction of nitrile **26** with lithium aluminum hydride 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 analogues **37a–d** were prepared in the same manner as described for the synthesis of compound **33** (Scheme 3). Nucleophilic substitution of bromide **6** with primary amines and subsequent deprotection provided the 5-benzylamine analogues **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 isolation of the 4′-benzylamine analogues **41a,b**. Addition of ethylmagnesium bromide and phenylmagnesium bromide to 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**.

Biological assays

The newly synthesized triclosan analogues were evaluated for their inhibitory activity (MIC_{50}) against *T. gondii* tachyzoites (measured as diminished uptake of [^{3}H]uracil or fluorescence with YFP-transfected parasites [see the Experimental Section below], Table 1). The toxicity toward human foreskin fibroblasts (HFF), in which the tachyzoites were cultured and compounds were tested, was determined by using the highest test compound concentration (10 μM) on host cells with 10% DMSO (*v*/*v*) as a control, and assessing the replication of non-confluent HFF in a [^{3}H]thymidine assay. The compounds were also screened in duplicate at a concentration of 1 μM for inhibition of *Tg*ENR enzyme activity. Analogues with significant inhibition (typically > 90% at 1 μM) were subsequently assayed in triplicate at 11 concentrations to determine their IC_{50} values. The in vitro parasite, human host cell, and enzyme data along



Scheme 2. Synthesis of 5- and 4′-isoxazole analogues of triclosan. *Reagents and conditions*: a) liquid H_2O/EtOH/ice (1:1:2), $H_2NOH{\cdot}HCl$, 50% aq NaOH, RT, 75 min, 90%; b) NCS, DMF, RT, 1.5 h, 100%; c) sodium ascorbate, $CuSO_4{\cdot}5H_2O$, $KHCO_3$, 1-alkyne, *t*BuOH/H_2O (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) Bu_4NI, CH_2Cl_2, RT, 5 min, then BCl_3, −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%.

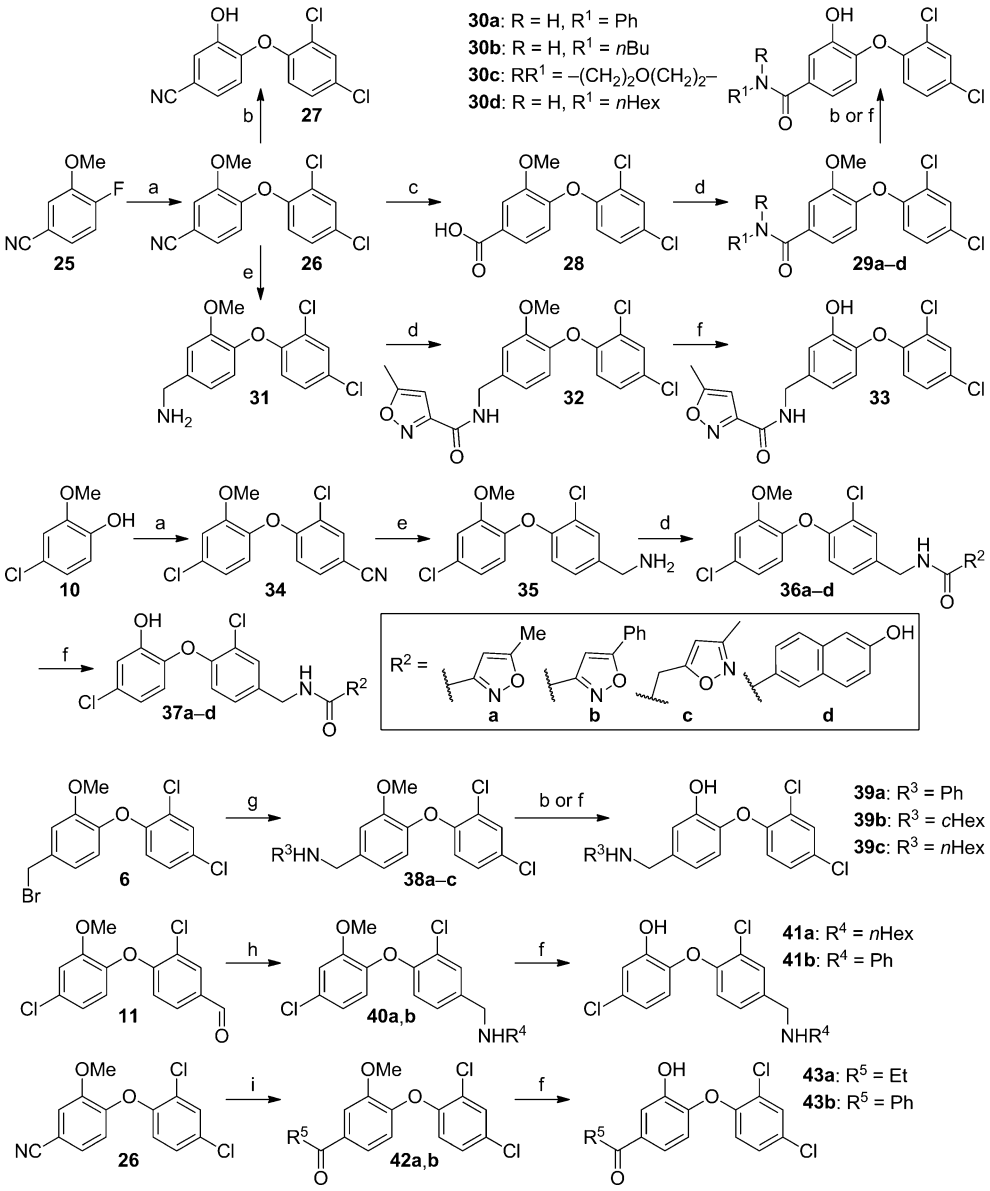
with predictions for the selected ADMET parameters are listed in Table 1.

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 hydrogen bonding between the triclosan hydroxy group, the ether linkage, the conserved tyrosine residue, and the 2′-hydroxy group 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 hydroxy 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 a decrease in the van der Waals forces and creates more space in the enzyme's binding pocket which, in turn, provides an 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 toward the inhibitor's entry portal; therefore, modification of this position with bulky substituents might allow an increase in enzyme–inhibitor interactions while improving inhibitor properties such as solubility, work that has been described earlier.[54]

While modifying the triclosan scaffold with various groups, we also paid attention to the drug-like properties of these new analogues. 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 analogues, ADMET prediction software (from ACD/Labs) was used to select compounds with improved permeability, water solubility, and other important physicochemical parameters, such as Clog *P*.

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 analogues in this series against *T. gondii* tachyzoites. On average, **16a** and **16c** were fourfold more active than triclosan (Table 1). Both compounds also showed high inhibition of *Tg*ENR enzyme activity (90 and 91%, respectively, at 1 μM compound concentration) with IC_{50} values similar to that of triclosan (43 and 26 nM, respectively, vs. 17 nM for triclosan). The ADMET suite predicted compound **16c** to possess improved Clog *P*, water solubility (S_w), 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 than those of **16c**. The improved drug-like properties along with high in vitro activity make analogue **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 analogues with attached cyclohexylamine (**39b**) and hexylamine (**39c**) substituents at the 5-position displayed activity similar to or better than that of triclosan (MIC_{50} = 0.25 μM, Table 1) in the parasite-based assay. Compounds **16b** and **37a** moderately inhibited the enzyme to the extent of 82 and 88%, respectively, at 1 μM. However, the remaining amine analogues 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 analogues may owe their activity in the parasite assay to interaction with targets other than *Tg*ENR.

The growth of tachyzoites was only weakly inhibited (MIC_{50} = 10 μM) by compounds bearing the corresponding unsubstituted triazole (**9c**), 5-methylisoxazole (**33**) and aniline (**39a**) groups at position 5. Additionally, derivatives **24a**,**b** 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 1 μM), and its IC_{50} was 19 nM, which was one of the lowest values observed for this series of analogues. In addition, the favorable predictions of the physicochemical properties of compound **33** such as Clog *P*, water solubility, and Caco-2 permeability make this molecule a viable lead for further rounds of medicinal chemistry optimization. Analogues **9c** and **39a**



Scheme 3. Substitution of triclosan at positions 5 and 4′ with cyano, carbamoyl, acyl, aminomethyl, and (acylamino)methyl groups. *Reagents and conditions*: a) 1. for **26**: 2,4-dichlorophenol, Cs_2CO_3, DMF, 120 °C, 21 h, 65%, 2. for **34**: 3-chloro-4-fluorobenzonitrile, Cs_2CO_3, DMF, 100 °C, 16 h, 75%; b) Bu_4NI, CH_2Cl_2, RT, 5 min, then BCl_3, −78 °C, 15 min, then RT, 2 h, for **27**: 82%; for **30a**: R = H, R^1 = Ph, 51%, for **30b**: R = R^1 = *n*Bu, 70%, for **30c**: RR^1 = –$(CH_2)_2O(CH_2)_2$–, 51%, for **39a**: R^3 = Ph, 38%, for **39b**: R^3 = *c*Hex, 20%, for **43a**: R^5 = Et, 11%, for **43b**: R^5 = Ph, 13%; c) 25% aq NaOH, EtOH, reflux, 21 h, 67%; d) 1. carboxylic acid, DIPEA, RT, 10 min, 2. BOP-Cl, NHR^{1}R^2, DIPEA, RT, 3. 24 h, for **29a**: R = H, R^1 = Ph, 81%, for **29b**: R = R^1 = *n*Bu, 100%, for **29c**: RR^1 = –$(CH_2)_2O(CH_2)_2$–, 77%, for **29d**: R = H, R^1 = *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. $LiAlH_4$, Et_2O, 0 °C, 0.5 h then RT, 3 h 2. H_2O, 1.0 M NaOH, for **31**: 81%, for **35**: 95%; f) BBr_3, CH_2Cl_2, −78 °C→RT within 1 h, then RT, 3 h, for **30d**: R = H, R^1 = *n*Hex, 69%, for **33**: 76%, for **37a**: 62%, for **37b**: 66%, for **37c**: 37%, for **37d:** 52%, for **39c**: R^3 = *n*Hex, 47%, for **41a**: R^4 = *n*Hex, 55%, for **41b**: R^4 = Ph, 74%; g) RNH_2, K_2CO_3, reflux, 24 h, for **38a**: R^3 = Ph, 48%, for **38b**: R^3 = *c*Hex, 54%, for **38c**: R^3 = *n*Hex, 30%; h) 1. RNH_2, powdered molecular sieves (4 Å), CH_2Cl_2, RT, 6–8 h; 2. $NaBH(OAc)_3$, RT, 24 h, for **40a**: R^4 = *n*Hex, 43%, for **40b**: R^4 = Ph, 85%; i) 1. R^5MgBr, 0 °C, 0.5 h, then RT, 18 h, 2. 1 M HCl for **42a**: R^5 = Et, 80%, for **42b**: R^5 = Ph, 68%.

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 μM), and their IC_{50} values were in the range of 24–54 nM (Table 1). The same trend was observed for the analogues with 5-phenylisoxazole (**37b**), (3-methylisoxazo-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 underscores the fine balance between increasing inhibitor solubility whilst not affecting its ability to cross the parasite cell membrane. Reasonable activity against purified *Tg*ENR was observed for an analogue bearing the 6-hydroxynaphthalene group at the 4′-position (**37d**). Nonetheless, its high Clog*P* 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 at inhibiting parasite whole-cell growth; therefore, these analogues are considered to be poor *Tg*ENR inhibitors. The triclosan analogues 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 (MIC_{50} = 7 μM, Table 1). Disappointingly, all the amide-modified analogues **30a–d** showed no 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 a hydroxy group on ring A.

Table 1. Activity data and ADMET predictions for triclosan-based *Tg*ENR inhibitors.

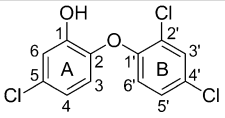
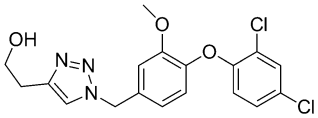
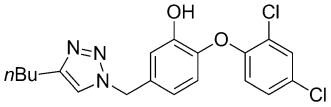
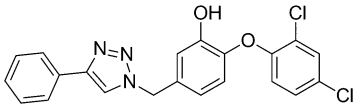
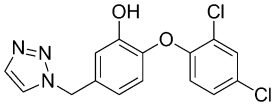
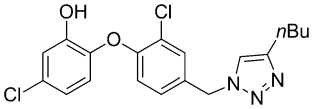
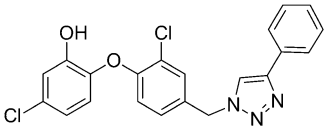
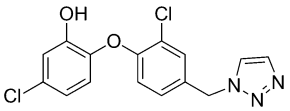
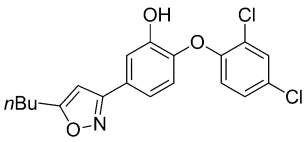
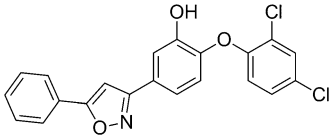
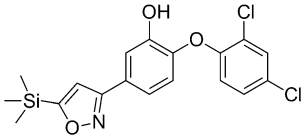
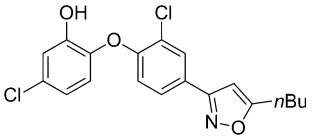
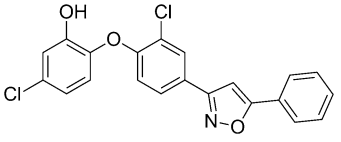
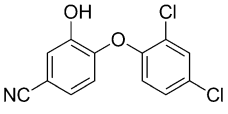
Compound		MIC_{50} [μM][a]	HFF [μM][a]	Inhib. [%][b,c]	IC_{50} [nM][b]	95% CI [nM][b,d]	Clog P[e]	vTPSA [Å^2][f]	S_w [10^{-3} mg mL^{-1}][g]	$F(>70)$[h]	Caco-2 [10^{-6} cm s^{-1}][i]
triclosan		3	>10	97	17	13–22	5.5	29.5	4.6	0.21	184
8d		>10	>10	12	ND		3.1	69.4	26	0.59	235
9a		>10	>10	**94**	38	30–48	5.5	60.2	0.9	0.04	214
9b		>10	>10	**93**	54	43–68	5.7	60.2	0.5	0.04	164
9c		10	>10	**93**	130	87–206	3.6	60.2	14	0.19	233
16a		**0.25**	>10	**90**	43	35–54	5.8	60.2	0.6	0.04	206
16b		*1*	>10	82	ND		6.0	60.2	0.8	0.04	152
16c		**0.25**	>10	**91**	**26**	23–41	*3.9*	60.2	10	0.19	235
20a		>10	>10	59	ND		6.6	55.5	0.3	0.04	80
20b		>10	>10	40	ND		6.9	55.5	0.1	0.21	68
20c		7	>10	25	ND		7.4	55.5	0.7	0.21	69
24a		8	>10	**92**	**18**	14–24	6.8	55.5	0.3	0.04	67
24b		10	>10	88	28	22–36	7.0	55.5	0.1	0.21	65
27		*2*	>10	**97**	**24**	16–36	4.6	53.2	1.1	0.78	233

Table 1. (Continued)

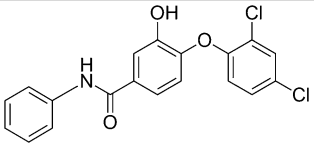
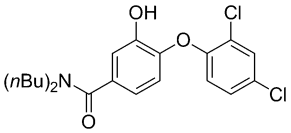
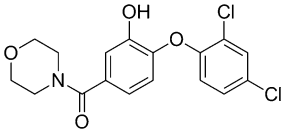
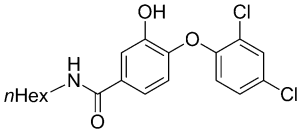
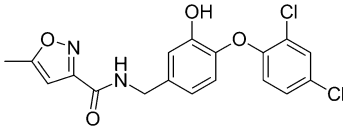
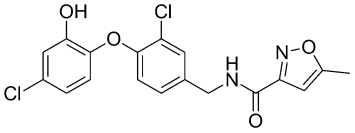
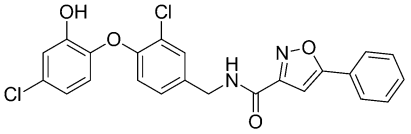
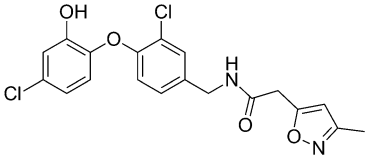
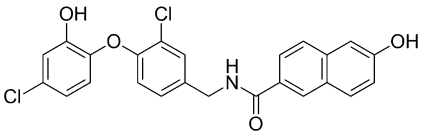
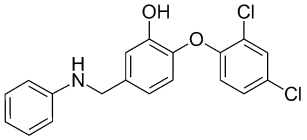
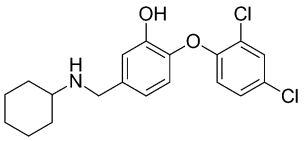
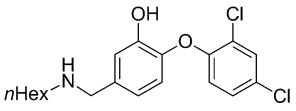
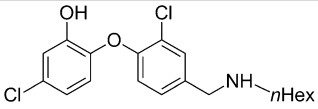
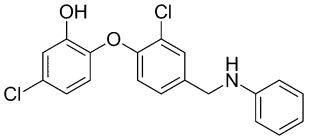
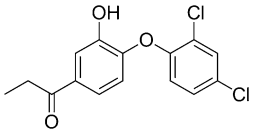
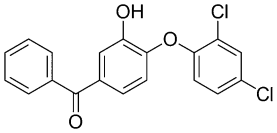
	Compound	MIC_{50} [μM][a]	HFF [μM][a]	Inhib. [%][b,c]	IC_{50} [nM][b]	95% CI [nM][b,d]	Clog P[e]	vTPSA [Å^2][f]	S_w [10^{-3} mg mL^{-1}][g]	$F(>70)$[h]	Caco-2 [10^{-6} cm s^{-1}][i]
30a		>10	>10	7	ND		5.4	58.6	0.1	0.20	118
30b		>10	>10	44	ND		6.7	49.8	1.0	0.04	183
30c		>10	>10	43	ND		3.7	59.0	28	0.06	235
30d		>10	>10	30	ND		6.2	58.6	0.4	0.21	61
33		10	ND	**96**	**19**	17–21	4.3	84.6	4.9	0.21	215
37a		*5*	ND	88	100	79–126	4.6	84.6	3.9	0.21	210
37b		>10	ND	80	ND		6.4	84.6	0.2	0.21	99
37c		>10	ND	**96**	**33**	27–40	*3.6*	84.6	4.1	0.29	201
37d		>10	ND	89	ND		6.3	78.8	0.1	0.04	42
39a		10	>10	**96**	130	98–174	5.5	41.5	0.3	0.04	48
39b		*4*	>10	34	ND		5.9	41.5	0.7	0.04	68
39c		*3*	10–1	16	ND		6.5	41.5	0.7	0.04	9

Table 1. (Continued)

	Compound	MIC_{50} [μM][a]	HFF [μM][a]	Inhib. [%][b,c]	IC_{50} [nM][b]	95% CI [nM][b,d]	Clog P[e]	vTPSA [Å^2][f]	S_w [10^{-3} mg mL^{-1}][g]	$F(>70)$[h]	Caco-2 [10^{-6} cm s^{-1}][i]
41a		*1*	>10	25	ND		6.8	41.5	0.4	0.04	6
41b		>10	>10	**94**	**31**	26–37	5.8	41.5	0.2	0.04	37
43a		>10	>10	85	380	300–481	5.0	46.5	1.3	0.21	233
43b		>10	>10	78	750	487–1150	6.1	46.5	0.2	0.20	136

[a] Parasite tissue culture assay (see Experimental Section for details; HFF = human foreskin fibroblasts). MIC_{50}: concentration required to yield half the RFU value 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 boldface; the next best are highlighted in italics. In addition, data from two to four separate experiments performed at different times using the compounds that fell in the >1–<10 μM range and two in the >0.1–<1 μM range were tested. Concentrations used 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. [b] *Tg*ENR enzyme assay (enzyme concentration: 5 nM). Data of highest activity are indicated in boldface; in the first column for ADMET (Clog *P*), compounds selected for testing in the murine model are highlighted in italics. [c] Percent inhibition at 1 μM compound concentration. [d] 95% confidence interval. [e] Calculated with ChemDraw Ultra 7.0. [f] Topological polar surface area. [g] Solubility in water. [h] Probability of bioavailability greater than 70%. [i] Permeability through Caco-2 monolayer.

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 analogues 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 toward the NAD^+ cofactor, which allows the two components to interact via π-stacking in the same manner observed with triclosan. The phenolic hydroxy group on ring A is in close proximity to the conserved Tyr189 residue and the 2′-hydroxy group of NAD^+, and thus they interact through hydrogen bonding. The ether linkage is beyond the hydrogen bond distance from the 2′-hydroxy group of NAD^+, and is therefore 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, 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 groups of Asn130 and Gly131. These new hydrogen bonding interactions of compound **16c** with surrounding *Tg*ENR residues may be decisive for its high inhibitory activity in the enzyme-based assay. Alternatively, molecular modeling revealed that the triazole group might rotate and take a position that is favorable for hydrogen bonding interaction with Ala231. Additionally, desirable in silico ADMET properties may explain the high activity of this analogue in the parasite-based assay.

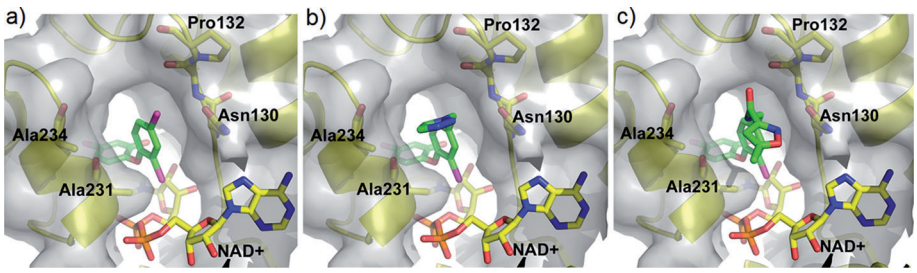


Figure 1. a) Crystal structure of the *Tg*ENR–NAD^+–triclosan complex with docked compounds b) **16c** and c) **37c**. The key residues that line the inhibitor binding pocket are shown in stick format along with the NAD^+ cofactor (yellow carbon atoms) and inhibitor (green carbon atoms). Oxygen atoms are in red, nitrogen in blue, phosphorus in orange, and chlorine is shown in magenta. The image was produced using PyMOL.

The two 4′-triazole-modified compounds **16a** and **16b** bind in a mode very similar to that of analogue **16c**, with higher IC_{50} values. This is attributed to the presence of substituents at the 4-position of the triazole ring.

The 4-butyltriazole **16a** had IC_{50} 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 *Tg*ENR was inhibited only by 82% at a compound concentration of 1 μM. 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 observed with triclosan. Additionally, the hydrogen bonds between the triazole group, Asn130, and Gly131 are missing, which explains the decreased activity of this inhibitor.

Derivative **33**, bearing a 5-methylisoxazole group at the 5-position, displayed excellent activity against recombinant *Tg*ENR. This compound inhibited the enzyme by 96% at 1 μM, and its IC_{50} value 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 is therefore able to interact via π-stacking with the NAD^+ cofactor. Moreover, the amide oxygen atom of **33** is hydrogen bonded to the 2′-hydroxy group of NAD^+, which provides additional stabilization for this moiety in the active site of the protein. The isoxazole methyl substituent points outward in the same direction as the chlorine atom on ring A of triclosan and thus might efficiently fill the hydrophobic cavity in the substrate binding site. The phenolic ring A of **33** is significantly shifted and is situated almost perpendicular relative to the original position of ring B of triclosan when bound to the enzyme. Nonetheless, its hydroxy group contributes to the hydrogen bonding interactions with the carbonyl oxygen atom 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 analogue. This apparent reverse binding mode was also observed for **37b**, which, in possessing an aromatic substituent on the isoxazole ring, also has a significantly bulky extension on the A ring. Because the only compounds that display this apparent reverse binding mode contain large extensions on the A ring, it is likely that the modeling does not take into account the flexibility within the binding site.[33,55] 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 that of triclosan. Although without a co-crystal structure, we cannot 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**, obviating the need for a reverse binding mode. Moreover, docking of a larger compound that contains bulky substitutions to both the A and B rings has shown that this reverse-mode binding is not required for activity.[77] Compounds **37a** and **37c** have a smaller methyl substituent on the isoxazole ring than **37b**, and are predicted to bind to the enzyme in the same fashion as triclosan (Figure 1c). Their aromatic rings A are involved in π-stacking and hydrogen bonding interactions in the active site, while the isoxazole ring of each is positioned toward the inhibitor's entrance. Additional stabilization arises from the hydrogen bonds between the compound's amide oxygen atom and the amide NH groups of Asn130 and Gly131.

After identifying the molecular interactions defining the activity of the most potent compounds in this series (**16a**,**c** and **33**), we were interested in explaining the activity, or lack thereof, displayed by the other analogues. Triazole analogues **9a**–**c** interact with the enzyme through a different binding mode from that of 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 toward 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 (compounds **9a**,**b**) imposes significant positional change on ring A in the binding site. The phenolic ring A of compounds **9a** and **9b** is positioned perpendicular (relative to ring B of triclosan), with its hydroxy group directed toward Tyr189 and the nicotinamide ribose of NAD^+, and thus is able to interact with those units by hydrogen bonding. Although the unsubstituted triazole group of analogue **9c** also interacts through hydrogen 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 hydrogen bonding interactions with the proximal residues. As a result, the IC_{50} values of compounds **9a** and **9b** are approximately three- and twofold lower, respectively, than that of analogue **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 a reverse mode to that of triclosan, and the visible hydrogen bond interactions between the ketone oxygen, Asn130, and Gly131 do not seem to improve the inhibitory activity of these compounds. The amine analogues **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 relative to that of triclosan. The NH hinge is engaged in hydrogen bonding interactions with the carbonyl oxygen atom of Ala129 which affects the biaryl ether moiety alignment in the active site, although π-stacking between ring B and NAD^+ as well as hydrogen bonding between the ring A hydroxy 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 re-

sponsible for the high activity (IC_{50} = 31 nM) of this derivative. Although the isoxazole compounds **20a–c** as well as the amide analogues **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, thereby preventing these inhibitors from optimal accommodation in the enzyme's binding pocket. This hypothesis is partly supported by analogues **24a** and **24b**, the isoxazole rings of which are rigidly appended at the 4′-position of the scaffold, yet 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 was observed that neither of those analogues decreased the parasite burden at a dose at which triclosan was effective (10 mg kg^{-1}; Figures 2 and 3). However, at a higher dose (75 mg kg^{-1}) both compounds protected the mice by decreasing parasite burden (Figure 4). These compounds were tenfold 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^{-1}. For comparison, the reference compounds colchicine, labetalol, propranolol, and ranitidine had the following respective values for Caco-2 permeability: 0.1, 8.5, 51.1, and 0.4 × 10^{-6} cm s^{-1}.

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 analogue **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 analogues) is significantly decreased. This would explain the need for higher doses (i.e., 75 mg kg^{-1}) to observe the protective effect of this compound in infected mice (Figure 4).[56]

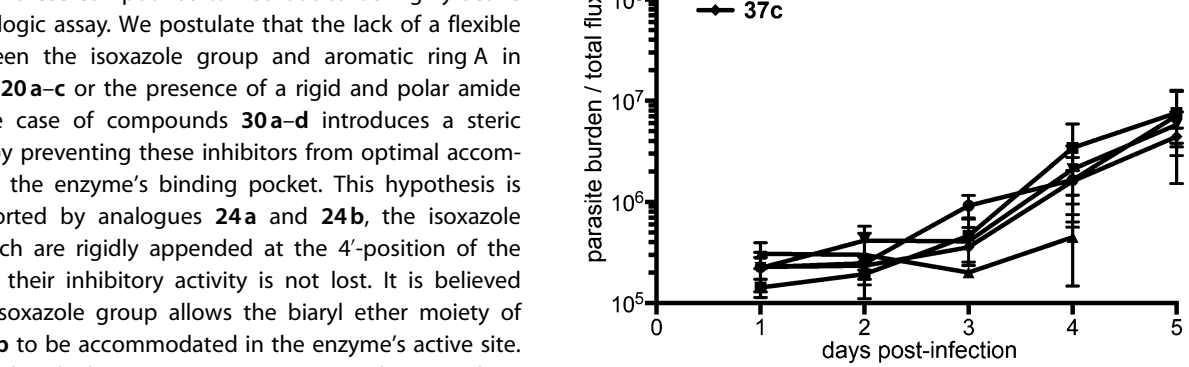


Figure 3. Comparison of triclosan and compounds **16c** and **37c** at 10 mg kg^{-1}. Compounds **16c** and **37c** had no effect on *T. gondii* in vivo at 10 mg kg^{-1}, a concentration at which triclosan demonstrated efficacy ($p > 0.05$).

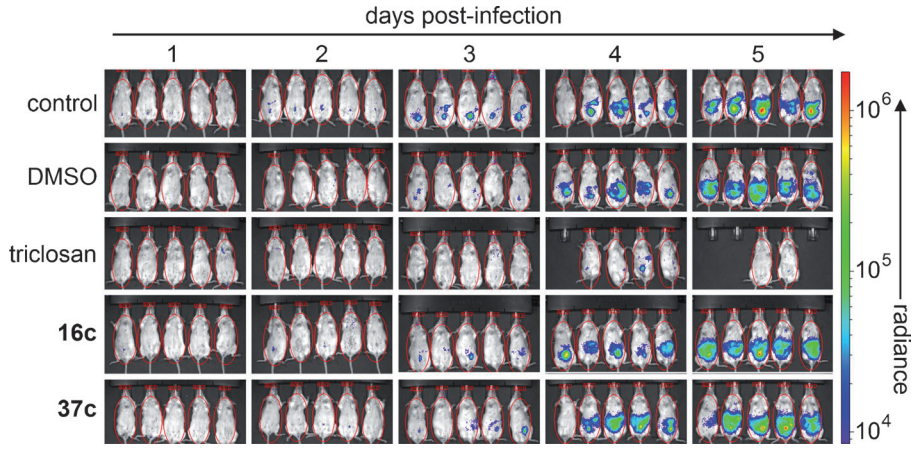


Figure 2. Xenogen camera images of mice showing the decreased effect of compounds **16c** and **37c** on parasite burden relative to triclosan, all dosed at 10 mg kg^{-1}.

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

It was previously shown that the FAS II pathway is nonessential for malaria during the erythrocytic stage.[75,76] In fact, these studies showed that the *Pf*ENR 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 analogues than strain TM91C235. The triazole analogue **9c** showed very modest activity against strain D6 of *P. falciparum*. Among the amide-modified compounds **30a–d**, only the analogue bearing the *N*,*N*-dibutyl substituent (**30b**) showed promising inhibitory activity. Those results indicate that aromatic (**30a**), rigid heterocycloaliphatic (**30c**), or even

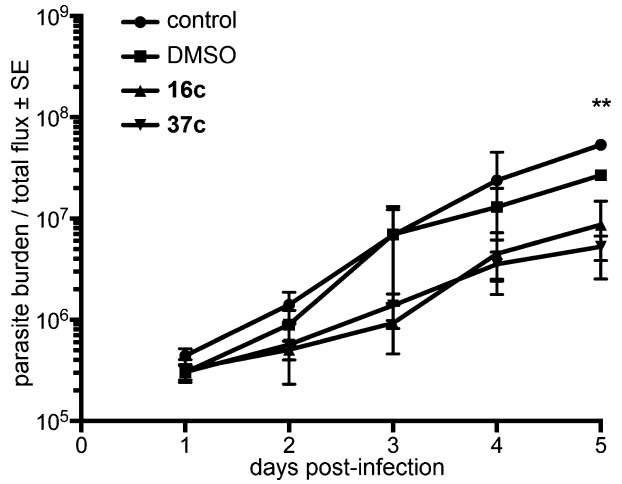


Figure 4. Compounds **16c** and **37c** are effective at decreasing parasite burden at 75 mg kg^{-1} five days after infection. This experiment was replicated twice. Although at 75 mg kg^{-1} **16c** and **37c** decreased parasite growth in vivo at day 5 post-infection ($p < 0.055$ and $^{**}p < 0.0079$ on days 4 and 5 by Mann–Whitney *U* test), this effect was not evident in mice dosed at 50 mg kg^{-1} (results not shown).

Table 2. Activity data for triclosan-based inhibitors of *P. falciparum* ENR.

Compd	SYBR Green D6[a]			SYBR Green C235[b]		
	IC_{50} [μM]	IC_{50} [ng mL^{-1}]	R^2	IC_{50} [μM]	IC_{50} [ng mL^{-1}]	R^2
9c	4.71	1583	0.87	ND[c]	> 2000	NA[d]
30a	ND	> 2000	NA	ND	> 2000	NA
30b	0.40	165.9	0.97	0.60	247	0.95
30c	ND	> 2000	NA	ND	> 2000	NA
30d	ND	> 2000	NA	ND	> 2000	NA
39a	4.56	1641	0.86	ND	> 2000	NA
39b	0.03	11.86	1.00	0.08	27.6	0.97
39c	2.75	856.5	0.93	ND	> 2000	NA
43a	2.75	856.5	0.93	ND	> 2000	NA
43b	ND	> 2000	NA	ND	> 2000	NA

[a] CDC/Sierra Leone strain of *P. falciparum*. [b] TM91C235 (WRAIR, Thailand) strain of *P. falciparum*. [c] ND: not determined. [d] NA: not active.

slightly longer aliphatic, such as *n*-hexyl (**30d**) substituents at position 5 seem to be detrimental for parasite inhibition. Notably, compound **30b** has the highest Clog*P* value among the tested **30a–d** analogues, which may imply that lipophilic molecules reach the molecular target more easily and therefore display better inhibitory activity. Similar levels of modest inhibitory activity were shown by compound **9c** and by the aniline analogue **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 IC_{50} value of 0.03 μM (Table 2). The high activity of compound **39b** against *P. falciparum* parasites makes this analogue one of the most potent triclosan derivatives possessing antimalarial activity reported to date. The other amine analogues **39a** and **39c** showed relatively weak antimalarial activity. The comparison of **39a** and **39b** is particularly illuminating, as these compounds display a difference of more than two 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 the *P. falciparum* strain TM91C235, only two compounds showed the desired inhibitory activity: the amide derivative **30b** and the amine analogue **39b**. Although those analogues 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 affect the same molecular target. Furthermore, given the poor *Tg*ENR inhibition by these compounds (44 and 34% at 1 μM, 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 attractive starting points 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, two had antiparasitic MIC_{50} values of 250 nM, which is approximately tenfold better than that of the lead compound, triclosan. Additionally, 14 analogues in this series had IC_{50} values ranging from 17 to 130 nM against the recombinant *Tg*ENR enzyme. Molecular docking revealed that the synthesized compounds can bind the target protein in a similar, or possibly reversed, mode relative to that of triclosan, depending on the nature of the substituents appended at the 5- and 4′-positions of the triclosan scaffold. The most promising analogues, **16c** and **37c**, were tested for their in vivo efficacy in the *T. gondii* mouse infection model, with limited efficacy. A small subset of these compounds was also evaluated against *P. falciparum* strains D6 and TM91C235. One analogue (**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 insight into the development of new inhibitors that target *Tg*ENR and an off-target in *P. falciparum*. The approach presented herein uses 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 the ENR enzyme in *T. gondii*.

Experimental Section

Molecular docking and ADMET calculations

ADMET calculations were performed with PhysChem, ADME, and Tox Suite (version 2012) provided by Advanced Chemistry Development Inc. (http://www.acdlabs.com). All calculation parameters were performed at default values of the predictor. Molecular docking studies were performed using FlexX (version 2.0.2 Linux64) provided by BioSolveIT GmbH (http://www.biosolveit.de/FlexX/index.html?ct=1). The X-ray crystal structure of *T. gondii* ENR in complex with triclosan was taken from the RCSB Protein Data Bank, PDB ID: 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 by using the bound triclosan as a guide within the *T. gondii* structure.

Biology

Inhibition of *Tg*ENR activity in vitro: Recombinant *Tg*ENR was purified as described previously.[57] An assay of *Tg*ENR enzyme activity used previously[37] was adapted for use in 96-well plates. The activity of *Tg*ENR was monitored by consumption of NADH ($\varepsilon_{340}=6220\ M^{-1}cm^{-1}$) with a SpectraMax M2 plate reader. Reactions were carried out in a final volume of 100 μL in 96-well Corning UV plates. Initially, 10 μL of 1 mM crotonyl-CoA (Sigma) was placed in each well with 1 μL DMSO (or compound dissolved in DMSO). Then a reaction mixture containing a final concentration of 5 nM *Tg*ENR, 100 mM sodium/potassium phosphate pH 7.5, 150 mM NaCl, and 100 μM 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 intervals of 35 s for 15 min. The first column of each plate contained blank reactions with 1% DMSO, which served as the baseline activity for each row of the assay plate. The last column of each plate contained the potent inhibitor triclosan in concentrations ranging from 10 μM to 610 pM (fourfold serial dilutions), 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 with those of the blanks in the first column of the plate. Compounds were initially screened in duplicate at a concentration of 1 μM. Compounds with significant inhibition (typically >90% at 1 μM) were further analyzed to determine IC_{50} values. These values were determined in triplicate, with each replicate consisting of 11 inhibitor concentrations ranging from 10 μM to 170 pM (threefold serial dilutions). Nonlinear regression analysis was performed using GraphPad Prism software. The *Z*-factor for this assay[58] was calculated from data spanning 25 plates, and was found to be 0.65.

Inhibition of *T. 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 3500 parasites per well. Parasites were allowed to infect cells for 1 h before the addition of inhibitors and control media. After 72 h, parasite proliferation was assessed by a [^{3}H]uracil incorporation assay,[59] or a YFP fluorescence assay, in which relative fluorescence of parasite samples was measured with 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] The effect of compounds on the replication of *T. gondii* within infected fibroblasts was determined by using assays described previously.[21,23,37,59–72] The primary goal of these studies was to identify compounds that are either inferior 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 concentrations of 10, 1, 0.1 and 0.01 μM. Those compounds that inhibited replication by 50% between 1 and 10 μM were tested at 1.25, 2.5, 5, and 10 μM. Those compounds that inhibited replication by 50% between 0.1 and 1 μM were tested at 0.1, 0.25, 0.5, and 0.75 μM. 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 were calculated. A curve displaying the results was made, allowing direct visual comparison of the data between experiments. Independent biological replicate experiments were performed at least two separate times for each compound to confirm the results were reproducible and consistent. Inhibitory index was calculated as: $[RFU_{(compound)}-RFU_{(control\ fibroblasts)}]/[RFU_{(DMSO\ control)}-RFU_{(control\ fibroblasts)}]\times 100$. MIC_{50} is defined as the compound concentration required to inhibit replication by 50%. This was determined by 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 are also presented for toxicity assays assessing the 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" 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 [^{3}H]thymidine incorporation assays using non-confluent HFF cells, 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 h, absorbance at λ 420 nm was measured using a Synergy H4 Hybrid Reader (BioTek) and Gen5 1.10 software.

Inhibition of *P. 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 antimalarial drugs or experimental compounds. As the intercalation of SYBR green I dye and its resulting fluorescence is proportional to parasite growth, a test compound that inhibits the growth of the parasite will result in 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 twofold serial dilutions of each test compound or mefloquine hydrochloride (Sigma–Aldrich, Cat. # M2319) suspended in DMSO. The final concentration range tested was 0.5–10000 ng mL^{-1} for all assays. Pre-dosed plates were stored at 4 °C until use, not to exceed five days. No difference was observed in drug sensitivity determinations between stored or fresh drug assay plates (data not shown). A batch control plate using chloroquine (Sigma–Aldrich, Cat. # C6628) at a final concentration of 2000 ng mL^{-1} was used to validate each assay run. The Tecan Freedom Evo liquid handling system (Tecan US Inc., Durham, NC, USA) 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 pre-dosed 384-well microtiter drug assay plates in culture volume of 38 μL 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% CO_2, 5% O_2, and 90% N_2 for 72 h. Lysis buffer (38 μL per well), consisting of 20 mM Tris·HCl, 5 mM EDTA, 1.6% Triton X, 0.016% saponin, and SYBR green I dye at 20× concentration (Invitrogen, Cat. # S-7567) was then added to the assay plates for a final SYBR green concentration of 10×. 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 h and examined for the relative fluorescence units (RFU) per well using a Tecan Genios Plus instrument (Tecan US). Each drug concentration was transformed into log[*X*] and plotted against the RFU values. The 50 and 90% inhibitory concentrations (IC_{50} and IC_{90} values) were then generated with GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) 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.

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 and ^{13}C NMR spectra were recorded on a Bruker spectrometer at 400 and 100 MHz, respectively, and were referenced to the residual peaks of $CHCl_3$ at $\delta = 7.26$ ppm or [D_6]DMSO at $\delta = 2.50$ ppm (^1H NMR), and $CDCl_3$ at $\delta = 77.23$ ppm or [D_6]DMSO at $\delta =$ 39.51 ppm (^{13}C NMR). Chemical shifts are reported in parts per million downfield of TMS, and the following abbreviations are 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 LCMS MSD (Hewlett Packard). HRMS experiments were performed on a Shimadzu LCMS-IT-TOF spectrometer. TLC was performed on Merck 60 F_{254} 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 λ 254 and 280 nm on a Shimadzu SPD-10A VP detector. Method I: flow rate: 17 mL min^{-1}, gradient: 25→100% MeOH in H_2O within 40 min; Method II: flow rate: 17 mL min^{-1}, gradient: 8→100% MeOH in H_2O 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 80 A column, with detection at λ 254 nm on a variable wavelength detector G1314A. Method I: flow rate: 1.4 mL min^{-1}, gradient: 30→100% MeOH in H_2O within 16 min (both solvents containing 0.05 vol% CF_3COOH); Method II: flow rate: 1.4 mL min^{-1}, gradient: 10→100% MeOH in H_2O within 21 min (both solvents containing 0.05 vol% CF_3COOH).

General Procedure A: Preparation of 1,3-disubstituted triazoles: The compounds were prepared by following a published 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 H_2O, 0.050 mmol, 0.1 equiv), $CuSO_4$ (1.25 mg freshly dissolved in 50 μL of deionized H_2O, 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 H_2O (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 H_2O (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*Bu_4NI–BCl_3**: A published procedure was followed.[47] In a 25 mL round-bottom flask were placed the starting material (0.50 mmol, 1.0 equiv), *n*BuNI (462 mg, 1.25 mmol, 2.50 equiv), and dry CH_2Cl_2 (4 mL). After stirring for 5 min at room temperature, the reaction mixture was cooled to −78 °C, and a solution of BCl_3 (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 H_2O (~5 mL), and stirring was continued for a further 30 min. The mixture was poured into saturated aqueous solution of $NaHCO_3$ (25 mL), and the products were extracted into CH_2Cl_2 (2×25 mL). The combined organic phases were washed with H_2O (25 mL), dried over Na_2SO_4, 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 BBr_3**: A published 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 CH_2Cl_2 (to prepare a 0.1 M solution). The flask was cooled to −78 °C, and BBr_3 (1.0 M solution in CH_2Cl_2, 5 equiv) was added dropwise. The reaction mixture was warmed gradually to room temperature within 1 h, and stirring was continued for a further 2.5–3 h. The reaction was terminated by the addition of H_2O, and the products were extracted into CH_2Cl_2 (2×25 mL). The combined organic phases were dried over Na_2SO_4, 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 by following a published procedure.[45] To the stirred solution of a carboximidoyl chloride (792 mg, 2.0 mmol) in *t*BuOH/H_2O (1:1, 4 mL) was added an alkyne (1.0 equiv) followed by sodium ascorbate (1.0 M solution in deionized H_2O, 200 μL, 10 mol%), and copper(II) sulfate pentahydrate (6 mg in 100 μL of deionized H_2O, 2 mol%). The reaction mixture was then treated with $KHCO_3$ (866 mg, 4.33 mmol) and left stirring for 1.5 h at room temperature before being poured onto H_2O (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 H_2O (30 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo to give crude material, which was used in the next step without additional purification.

4-(5,5-Dimethyl-1,3-dioxan-2-yl)-2-methoxyphenol (2): The title compound was prepared by following a published 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 held at reflux in dry toluene (25 mL) for 3 h. The reaction mixture was cooled to room temperature and poured into toluene/H_2O (1:1, 300 mL). The phases were separated, and the organic phase was washed with H_2O (150 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 (hexanes/EtOAc, 5:1–3:1) to give pure product as a white crystalline material (6.19 g, 87%). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; 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 by following a published 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 Cs_2CO_3 (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 H_2O (200 mL), and the products were extracted into EtOAc (3×100 mL). The combined organic phases were washed with H_2O (100 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to give crude material as a yellow solid, which was purified by recrystallization from CH_2Cl_2/EtOAc to give the pure product as a white crystalline material (3.90 g, 51%). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 98.7%; MS (ESI+) m/z 383 [$M+H^+$, $^{35}Cl_2$].

4-(2,4-Dichlorophenoxy)-3-methoxybenzaldehyde (4): The title compound was prepared by following a published 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 *para*-toluenesulfonate (1.15 g, 4.56 mmol, 0.38 equiv) was added. The reaction mixture was held at reflux for 2.5 h before cooling to room temperature. The solvent was evaporated in vacuo, and the residue dissolved in Et_2O (100 mL). The organic phase was washed with saturated aqueous solution of $NaHCO_3$ (100 mL) and brine, dried over Na_2SO_4, 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. ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 98.3%.

[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl]methanol (5): The title compound was prepared by following a published 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 $NaBH_4$ (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 NH_4Cl (25 mL). The mixture was poured into saturated aqueous solution of NH_4Cl/EtOAc (1:1, 100 mL), and the products were extracted into EtOAc (2×100 mL). The combined organic phases were washed with H_2O (100 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to give crude material as a pale-yellow oil, which was purified by flash chromatography (hexanes/EtOAc, 5:1–3:1) to give the pure product as a pale-yellow oil (3.03 g, 84%). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 98.9%; MS (ESI+) m/z 281 [$M+H^+-H_2O$, $^{35}Cl_2$].

4-(Bromomethyl)-1-(2,4-dichlorophenoxy)-2-methoxybenzene (6): The title compound was prepared by following a modified published 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 CBr_4 (4.97 g, 15.0 mmol, 1.5 equiv) and PPh_3 (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 hexanes/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%). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 91.9%; MS (ESI+) m/z 281 [M^+-Br, $^{35}Cl_2$].

4-(Azidomethyl)-1-(2,4-dichlorophenoxy)-2-methoxybenzene (7): This compound was prepared by following a published 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 NaN_3 (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 H_2O (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%). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 98.6%; MS (ESI+) m/z 281 [M^+-N_3, $^{35}Cl_2$].

4-Butyl-1-[4-(2,4-dichlorophenoxy)-3-methoxybenzyl]-1*H*-1,2,3-triazole (8a): The title compound was prepared by following General Procedure A with 1-hexyne as the alkyne (pale-yellow oil, 165 mg, 86% on a 0.47 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 87.6%; MS (ESI+) *m/z* 406 [$M+H^+$, $^{35}Cl_2$].

1-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]-4-phenyl-1*H*-1,2,3-triazole (8b): The title compound was prepared by following General Procedure A with phenylacetylene as the alkyne (pale-yellow solid, 213 mg, 100%). ^{1}H NMR ([D$_6$]DMSO): $\delta=8.66$ (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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=151.9$, 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 ppm; HPLC purity 90%; MS (ESI+) *m/z* 426 [$M+H^+$, $^{35}Cl_2$].

1-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]-4-(trimethylsilyl)-1*H*-1,2,3-triazole (8c): The title compound was prepared by following General Procedure A with trimethylsilylacetylene as the alkyne (pale-yellow solid, 571 mg, 97% on a 1.5 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=8.24$ (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 ppm (s, 9H); ^{13}C NMR ([D$_6$]DMSO): $\delta=151.9$, 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 ppm (3C); HPLC purity 86.1%; MS (ESI+) *m/z* 422 [$M+H^+$, $^{35}Cl_2$].

2-[1-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]-1*H*-1,2,3-triazol-4-yl]ethanol (8d): The title compound was prepared by following General Procedure A with 4-butyn-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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=7.95$ (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 ppm (t, $J=6.9$ Hz, 2H); ^{13}C NMR ([D$_6$]DMSO): $\delta=152.0$, 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 ppm; HPLC purity 97.0%; MS (ESI+) *m/z* 394 [$M+H^+$, $^{35}Cl_2$].

5-[(4-Butyl-1*H*-1,2,3-triazol-1-yl)methyl]-2-(2,4-dichlorophenoxy)-phenol (9a): The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=$ 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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=152.2$, 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 ppm; HPLC purity 99.7%; MS (ESI+) *m/z* 392 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{19}H_{20}Cl_2N_3O_2$ 392.0927 [$M+H^+$, $^{35}Cl_2$], found 392.0924.

2-(2,4-Dichlorophenoxy)-5-[(4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl]phenol (9b): The title compound was prepared by following 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%). ^{1}H NMR ([D$_6$]DMSO): $\delta=9.91$ (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 ppm (s, 2H); ^{13}C NMR ([D$_6$]DMSO): $\delta=152.2$, 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 ppm; HPLC purity 98.4%; MS (ESI+) *m/z* 412 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{21}H_{16}Cl_2N_3O_2$ 412.0614 [$M+H^+$, $^{35}Cl_2$], found 412.0596.

2-(2,4-Dichlorophenoxy)-5-(1*H*-1,2,3-triazol-1-ylmethyl)phenol (9c): 1-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]-1*H*-1,2,3-triazole was prepared according to a published procedure.[46] To a stirred solution of **8c** (0.5 mmol) in dry THF (3.0 mL) was added dropwise at room temperature *n*Bu$_4$NF (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%). ^{1}H NMR ([D$_6$]DMSO): $\delta=8.23$ (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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=152.0$, 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 ppm; HPLC purity 98.4%; MS (ESI+) *m/z* 350 [$M+H^+$, $^{35}Cl_2$]. **Compound 9c was then prepared by following 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 mmol scale). ^{1}H NMR (DMSO): $\delta=9.90$ (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 ppm (br s, 2H); ^{13}C NMR (DMSO): $\delta=152.2$, 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 ppm; HPLC purity 99.7%; MS (ESI+) *m/z* 336 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{15}H_{12}Cl_2N_3O_2$ 336.0301 [$M+H]^+$, found 336.0297.

3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzaldehyde (11): The title compound was prepared by following a published 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 (2.38 g, 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 H_2O. The phases were separated, and the aqueous phase was extracted with EtOAc (3×100 mL). The combined organic phases were washed with H_2O (3×100 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 3:1–3:2) to obtain the title compound as a pale-yellow solid (4.10 g, 92%). ^{1}H NMR ([D$_6$]DMSO): $\delta=9.88$ (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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=190.7$, 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 ppm; 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=7.46$ (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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=151.3$, 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 ppm; 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 96.2%.

4-Butyl-1-[3-chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-1*H*-1,2,3-triazole (15a): The title compound was prepared by following General Procedure A with 1-hexyne as the alkyne. Purification by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/MeOH 5:1–4:1) gave the pure product as a pale-yellow oil (324 mg, 80% on a 1.0 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 98.0%.

1-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-4-phenyl-1*H*-1,2,3-triazole (15b): The title compound was prepared by following General Procedure A with phenylacetylene as the alkyne. Purification by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/MeOH 5:1–4:1) gave the pure product as pale-yellow oil (343 mg, 80% on a 1.0 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 96.0%.

1-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-4-(trimethylsilyl)-1*H*-1,2,3-triazole (15c): The title compound was prepared by following General Procedure A with trimethylsilylacetylene as the alkyne. Purification by flash chromatography on silica gel (hexanes, then hexanes/EtOAc 3:1–3:2) gave the pure product as pale-yellow oil (319 mg, 82% on a 0.92 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 9H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm (3C); HPLC purity 96.3%; MS (ESI+) m/z 422 [$M+H^+$, $^{35}Cl_2$].

2-[4-[(4-Butyl-1*H*-1,2,3-triazol-1-yl)methyl]-2-chlorophenoxy]-5-chlorophenol (16a): The title compound was prepared by following General Procedure C with **15a** as the starting material. Purification by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/MeOH 5:1–4:1) followed by preparative HPLC gave the pure product as a white crystalline solid (142 mg, 48% on a 0.76 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 99.7%; HRMS (ESI+) calcd for $C_{19}H_{20}Cl_2N_3O_2$ 392.0927 [$M+H^+$, $^{35}Cl_2$], found 392.0932.

5-Chloro-2-[2-chloro-4-[(4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl]-phenoxy]phenol (16b): The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 2H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 98.6%; HRMS (ESI+) calcd for $C_{21}H_{16}Cl_2N_3O_2$ 412.0614 [$M+H^+$, $^{35}Cl_2$], found 412.0622.

5-Chloro-2-[2-chloro-4-[(1*H*-1,2,3-triazol-1-yl)methyl]phenoxy]-phenol (16c): 1-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-1*H*-1,2,3-triazole was used; the same procedure was applied as described above for the synthesis of compound **9c**. Purification by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/MeOH 5:1–4:1) gave the pure product as pale-yellow oil (190 mg, 76% on a 0.71 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 96.5%. **Compound 16c was then prepared by following General Procedure C** with 1-[3-chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]-1*H*-1,2,3-triazole as the starting material. Purification by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/MeOH 5:1–4:1) followed by preparative HPLC gave the pure product as a white solid (111 mg, 65% on a 0.51 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 2H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 99.6%; HRMS (ESI+) calcd for $C_{15}H_{12}Cl_2N_3O_2$ 336.0301 [$M+H^+$, $^{35}Cl_2$], found 336.0309.

(*E*)-1-[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl]-*N*-hydroxymethanimine (17): The title compound was prepared by following a published 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 NH_4OCl (0.49 g, 7.0 mmol, 1.0 equiv), followed by dropwise addition of NaOH (1.40 mL of a 50% solution in H_2O, 17.5 mmol, 2.5 equiv), while keeping the temperature $<30\,^{\circ}C$. After stirring at room temperature for 75 min, the reaction mixture was washed with Et_2O (30 mL). The aqueous phase was acidified to pH 6 by addition of concentrated HCl while keeping

the temperature $<30\,^{\circ}C$, and extracted with Et_2O (2×30 mL). The combined organic phases were dried over Na_2SO_4, 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 ([D$_6$]DMSO): $\delta=11.24$ (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 ppm (s, 3H); HPLC purity 85.8%.

4-(2,4-Dichlorophenoxy)-*N*-hydroxy-3-methoxybenzenecarboximidoyl chloride (18): The title compound was prepared by following a published 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 H_2O and extracted with Et_2O (2×50 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, 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 ([D$_6$]DMSO): $\delta=12.42$ (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 ppm (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 by following 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 by following 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 mmol scale). ^1H NMR ([D$_6$]DMSO): $\delta=10.08$ (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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=174.1$, 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 ppm; HPLC purity 98.0%; MS (ESI+) *m/z* 378 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{19}H_{18}Cl_2NO_3$ 378.0658 [$M+H^+$, $^{35}Cl_2$], found 378.0644.

2-(2,4-Dichlorophenoxy)-5-(5-phenyl-1,2-oxazol-3-yl)phenol (20b): The title compound was prepared by following 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 mmol scale). ^1H NMR ([D$_6$]DMSO): $\delta=10.16$ (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 ppm (d, $J=8.8$ Hz, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta=169.7$, 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 ppm; HPLC purity 97.4%; MS (ESI+) *m/z* 398 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{21}H_{14}Cl_2NO_3$ 398.0345 [$M+H^+$, $^{35}Cl_2$], found 398.0330.

2-(2,4-Dichlorophenoxy)-5-[5-(trimethylsilyl)-1,2-oxazol-3-yl]phenol (20c): The title compound was prepared by following 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 mmol scale). ^1H NMR ([D$_6$]DMSO): $\delta=10.08$ (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 ppm (s, 9H); ^{13}C NMR ([D$_6$]DMSO): $\delta=178.5$, 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 ppm (3C); HPLC purity 99.3%; MS (ESI+) *m/z* 394 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{18}H_{18}Cl_2NO_3Si$ 394.0428 [$M+H^+$, $^{35}Cl_2$], found 394.0431.

(*E*)-1-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)phenyl]-*N*-hydroxymethanimine (21): The title compound was prepared by following a published procedure.[45] The product was obtained as a pale-yellow solid in a yield of 0.70 g (90% on a 2.5 mmol scale) and was used directly in the next step. ^1H NMR ([D$_6$]DMSO): $\delta=11.28$ (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 ppm (s, 3H).

3-Chloro-4-(4-chloro-2-methoxyphenoxy)-*N*-hydroxybenzenecarboximidoyl Chloride (22): The title compound was prepared by following a published procedure.[45] The product was obtained as a yellow dense oil in a yield of 0.78 g (100% on a 2.25 mmol scale) and was used directly in the next step. ^1H NMR ([D$_6$]DMSO): $\delta=12.46$ (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 ppm (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 by following 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 mmol scale); phenylacetylene to give **23b** (yellow solid, 400 mg, 88% on a 1.1 mmol scale).

2-[4-(5-Butyl-1,2-oxazol-3-yl)-2-chlorophenoxy]-5-chlorophenol (24a): The title compound was prepared by following 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 mmol scale). ^1H NMR ([D$_6$]DMSO): $\delta=10.33$ (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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=174.3$, 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 ppm; HPLC purity 99.6%; MS (ESI+) *m/z* 378 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{19}H_{18}Cl_2NO_3$ 378.0658 [$M+H^+$, $^{35}Cl_2$], found 378.0663.

5-Chloro-2-[2-chloro-4-(5-phenyl-1,2-oxazol-3-yl)phenoxy]phenol (24b): The title compound was prepared by following 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 mmol scale). ^1H NMR ([D$_6$]DMSO): $\delta=10.36$ (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 ppm (d, $J=8.6$ Hz, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta=169.8$, 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 ppm; HPLC purity 99.2%; MS (ESI+) *m/z* 398 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{21}H_{14}Cl_2NO_3$ 398.0345 [$M+H^+$, $^{35}Cl_2$], found 398.0346.

4-(2,4-Dichlorophenoxy)-3-methoxybenzonitrile (26): The title compound was prepared by following a published 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 H_2O (75 mL), and extracted with EtOAc (2×75 mL). The combined organic phases were washed with H_2O (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 5:1–3:1) to give the pure product as pale-yellow oil (382 mg, 65%). ^{1}H NMR ([D$_6$]DMSO): $\delta=7.76$ (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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=150.2$ (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 ppm; HPLC purity 95.5%.

4-(2,4-Dichlorophenoxy)-3-hydroxybenzonitrile (27): The title compound was prepared by following General Procedure B with **26** as the starting material. The crude material was purified by flash chromatography on silica gel (hexanes, then hexanes/EtOAc 5:1–3:1) to give the pure product as a pale-yellow oil (230 mg, 82% on a 1.0 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=10.61$ (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 ppm (overlapping d, $J=8.9$ Hz, 1H); ^{13}C NMR ([D$_6$]DMSO): $\delta=150.6$, 148.9, 147.2, 130.0, 128.7, 128.1, 124.5, 124.3, 120.4, 120.3, 120.2, 118.5, 107.3 ppm; HPLC purity 99.3%; MS (ESI+) *m/z* 320 [$M+Na^+ + H_2O$, $^{35}Cl_2$]; HRMS (ESI−) calcd for $C_{13}H_6Cl_2NO_2$ 277.9781 [$M-H^+$, $^{35}Cl_2$], found 277.9771.

4-(2,4-Dichlorophenoxy)-3-methoxybenzoic acid (28): The title compound was prepared by following a published procedure.[37] Nitrile **26** (1.47 g, 5.0 mmol) was dissolved in EtOH (16 mL), and NaOH (2.60 mL of a 25% aqueous solution) was added. The reaction mixture was held at reflux 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 H_2O, and dried. This crude material was purified by flash chromatography on silica gel (hexanes/EtOAc 3:1–2:3) to give the pure product as a white solid (1.05 g, 67%). ^{1}H NMR ([D$_6$]DMSO): $\delta=13.03$ (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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=166.8$, 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 ppm; 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 by following a published procedure.[50] The crude product was purified by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/MeOH 10:1) to give the pure product as pale-yellow oil (220 mg, 81% on a 0.70 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=10.23$ (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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=164.5$, 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 ppm; HPLC purity 84.7%; MS (ESI+) *m/z* 388 [$M+H^+$, $^{35}Cl_2$].

4-(2,4-Dichlorophenoxy)-3-methoxy-*N*,*N*-dibutylbenzamide (29b): The title compound was prepared by following a published procedure.[50] The crude material was purified by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/MeOH 10:1–5:1) to give the pure product as a colorless oil (296 mg, 100% on a 0.70 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=7.71$ (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 ppm (br s, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=169.5$, 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 ppm; HPLC purity 94.4%; MS (ESI+) *m/z* 424 [$M+H^+$, $^{35}Cl_2$].

[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl](morpholin-4-yl)methanone (29c): The title compound was prepared by following a published procedure.[50] The crude material was purified by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/MeOH 7:1–4:1) to give the pure product as a colorless oil (296 mg, 77% on a 1.0 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=7.72$ (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 ppm (m, 8H); ^{13}C NMR ([D$_6$]DMSO): $\delta=$ 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 ppm; HPLC purity 91.0%; MS (ESI+) *m/z* 382 [$M+H^+$, $^{35}Cl_2$].

4-(2,4-Dichlorophenoxy)-*N*-hexyl-3-methoxybenzamide (29d): The title compound was prepared by following a published procedure.[50] The crude material was purified by flash chromatography on silica gel (hexanes, then hexanes/EtOAc 3:1–3:2) to provide the product as a colorless oil (199 mg, 42% on a 1.2 mmol scale), which contained 16% (by HPLC) of the starting material, the corresponding carboxylic acid. ^{1}H NMR ([D$_6$]DMSO): $\delta=8.47$ (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 ppm (t, $J=6.7$ Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=165.2$, 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 ppm; HPLC purity 83.4%.

4-(2,4-Dichlorophenoxy)-3-hydroxy-*N*-phenylbenzamide (30a): The title compound was prepared by following 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 mmol scale). ^{1}H NMR (DMSO): $\delta=10.20$ (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 ppm (d, $J=8.8$ Hz, 1H); ^{13}C NMR (DMSO): $\delta=164.8$, 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 ppm; HPLC purity 100%; MS (ESI+) *m/z* 374 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{19}H_{14}Cl_2NO_3$ 374.0345 [$M+H^+$, $^{35}Cl_2$], found 374.0334.

4-(2,4-Dichlorophenoxy)-3-hydroxy-*N*,*N*-dibutylbenzamide (30b): The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=$ 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 (brs, 2H), 3.17 (brs, 2H), 1.53–1.49 (m, 4H), 1.31 (brs, 2H), 1.12 (brs, 2H), 0.92 (brs, 3H), 0.75 ppm (brs, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=169.6$, 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 ppm; HPLC purity 100%; MS (ESI+) *m/z* 410 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{21}H_{26}Cl_2NO_3$ 410.1284 [$M+H^+$, $^{35}Cl_2$], found 410.1272.

[4-(2,4-Dichlorophenoxy)-3-hydroxyphenyl](morpholin-4-yl)methanone (30c): The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=10.07$ (brs, 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 ppm (m, 8H); ^{13}C NMR ([D$_6$]DMSO): $\delta=168.4$, 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 ppm (2C); HPLC purity 97.6%; MS (ESI+) *m/z* 368 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{17}H_{16}Cl_2NO_4$ 368.0451 [$M+H^+$, $^{35}Cl_2$], found 368.0445.

4-(2,4-Dichlorophenoxy)-*N*-hexyl-3-hydroxybenzamide (30d): The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=9.98$ (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 ppm (t, $J=6.7$ Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=165.4$, 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 ppm; HPLC purity 99.6%; HRMS (ESI+) calcd for $C_{19}H_{21}Cl_2NO_3$ 382.0971 [$M+H^+$, $^{35}Cl_2$], found 382.0978.

1-[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl]methanamine (31): The title compound was prepared by following a published procedure.[51] To a stirred suspension of $LiAlH_4$ (114 mg, 3.0 mmol) in dry Et_2O (20 mL) was added dropwise a solution of nitrile **26** (294 mg, 1.0 mmol) in dry Et_2O (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 H_2O (0.5 mL) and NaOH (0.5 mL of a 1.0 M solution). Et_2O was added and the mixture filtered. The filtrate was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give a pale-yellow oil, which was purified by flash chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/MeOH 5:1) to give the pure product as pale-yellow oil (242 mg, 81%). ^{1}H NMR ([D$_6$]DMSO): $\delta=7.67$ (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 ppm (s, 2H); ^{13}C NMR ([D$_6$]DMSO): $\delta=152.6$, 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 ppm; HPLC purity 95.1%.

***N*-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]-5-methyl-1,2-oxazole-3-carboxamide (32)**: The title compound was prepared by following a published procedure.[50] The crude material 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 pale-yellow dense oil (107 mg, 32% on a 0.81 mmol scale), which was used directly in the next step. ^{1}H NMR ([D$_6$]DMSO): $\delta=9.27$ (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 ppm (d, $J=0.8$ Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=171.3$, 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 ppm; HPLC purity 94.4%.

***N*-[4-(2,4-Dichlorophenoxy)-3-hydroxybenzyl]-5-methyl-1,2-oxazole-3-carboxamide (33)**: The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=9.76$ (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 ppm (brs, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=171.3$, 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 ppm; HPLC purity 99.7%; HRMS (ESI+) calcd for $C_{18}H_{15}Cl_2N_2O_4$ 393.0403 [$M+H^+$, $^{35}Cl_2$], found 393.0405.

3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzonitrile (34): The title compound was prepared by following the same procedure as described for the synthesis of compound **26**. Purification by recrystallization from MeOH provided the pure product as a pale-yellow solid (2.20 g, 75% on a 10 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=8.15$ (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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): $\delta=156.8$, 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 ppm; HPLC purity 94.5%.

1-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)phenyl]methanamine (35): The title compound was prepared by following the same procedure as described for the synthesis of compound **31**. The crude material 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 yellow oil (1.37 g, 86% on a 5.3 mmol scale). ^{1}H NMR ([D$_6$]DMSO): $\delta=7.51$ (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 ppm (s, 2H); ^{13}C NMR ([D$_6$]DMSO): $\delta=151.2$, 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 ppm; 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 by following a published 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 mmol scale), 5-phenylisoxazole-3-carboxylic acid to give **36b** as a pale-yellow oil (355 mg, 50% on a 1.50 mmol scale), 2-(3-methylisoxazol-5-yl)acetic acid to give **36c** as a yellow dense oil (500 mg, 79% on a 1.50 mmol scale), and 6-hydroxynaphthalene-2-carboxylic acid to give **36d** as an off-white solid (600 mg, 85% on a 1.50 mmol scale).

***N*-[3-Chloro-4-(4-chloro-2-hydroxyphenoxy)benzyl]-5-methyl-1,2-oxazole-3-carboxamide (37a)**: The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (d, J = 0.7 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 99.0%; HRMS (ESI+) calcd for $C_{18}H_{15}Cl_2N_2O_4$ 393.0403 [$M+H^+$, $^{35}Cl_2$], 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 by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (d, J = 6.0 Hz, 2H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 99.4%; HRMS (ESI+) calcd for $C_{23}H_{17}Cl_2N_2O_4$ 455.0560 [$M+H^+$, $^{35}Cl_2$], 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 by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 98.6%; HRMS (ESI+) calcd for $C_{19}H_{17}Cl_2N_2O_4$ 407.0560 [$M+H^+$, $^{35}Cl_2$], found 407.0546.

***N*-[3-Chloro-4-(4-chloro-2-hydroxyphenoxy)benzyl]-6-hydroxynaphthalene-2-carboxamide (37d)**: The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (d, J = 5.8 Hz, 2H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 97.7%; HRMS (ESI+) calcd for $C_{25}H_{17}Cl_2NO_4$ 454.0607 [$M+H^+$, $^{35}Cl_2$], 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 K_2CO_3 (207 mg, 1.5 mmol, 3.0 equiv) followed by aniline (1.5 mmol, 3.0 equiv). The mixture was held at reflux for 16 h, cooled to room temperature, poured into H_2O (30 mL), and extracted with Et_2O (2×20 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 3:1–3:2) to give the pure product as pale-yellow oil (90 mg, 48%). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 95%; MS (ESI+) m/z 281 [$M+H^+-PhNH_2$, $^{35}Cl_2$].

***N*-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]cyclohexanamine (38b)**: The title compound was prepared by 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (m, 5H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm (2C); HPLC purity 95.5%; MS (ESI+) m/z 380 [$M+H^+$, $^{35}Cl_2$].

***N*-[4-(2,4-Dichlorophenoxy)-3-methoxybenzyl]hexan-1-amine (38c)**: The title compound was prepared by 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 5:1–4:1) to give the pure product as a pale-yellow oil (115 mg, 30% on a 1.0 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (t, J = 6.9 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 96.6%; MS (ESI+) m/z 382 [$M+H^+$, $^{35}Cl_2$].

2-(2,4-Dichlorophenoxy)-5-[(phenylamino)methyl]phenol (39a): The title compound was prepared by following 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 mmol scale). ^{1}H 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 ppm (d, J = 6.0 Hz, 2H); ^{13}C 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 ppm; HPLC purity 99.4%; MS (ESI+) m/z 360 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{19}H_{16}Cl_2NO_2$ 360.0553 [$M+H^+$, $^{35}Cl_2$], found 360.0543.

5-[(Cyclohexylamino)methyl]-2-(2,4-dichlorophenoxy)phenol (39b): The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (m, 5H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm (2C); HPLC purity 100%; MS (ESI+) *m/z* 366 [$M+H^+$, $^{35}Cl_2$], HRMS (ESI+) calcd for $C_{19}H_{22}Cl_2NO_2$ 366.1022 [$M+H^+$, $^{35}Cl_2$], found 366.1006.

2-(2,4-Dichlorophenoxy)-5-[(hexylamino)methyl]phenol (39c): The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (t, J=6.9 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 100%; MS (ESI+) *m/z* 368 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{19}H_{24}Cl_2NO_2$ 368.1179 [$M+H^+$, $^{35}Cl_2$], found 368.1182.

***N*-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]hexan-1-amine (40a)**: The title compound was prepared by following a published procedure.[52] The product was obtained as a pale-yellow oil in a yield of 165 mg (43% on a 1.0 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (t, J=6.9 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 99.1%; MS (ESI+) *m/z* 369 [$M+H^+$, $^{35}Cl_2$].

***N*-[3-Chloro-4-(4-chloro-2-methoxyphenoxy)benzyl]aniline (40b)**: The title compound was prepared by following a published procedure.[52] The product was obtained as pale-yellow oil in a yield of 315 mg (85% on a 1.0 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 (fs 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 98.5%; MS (ESI+) *m/z* 361 [$M+H^+$, $^{35}Cl_2$].

5-Chloro-2-[2-chloro-4-[(hexylamino)methyl]phenoxy]phenol (41a): The title compound was prepared by following 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 mmol scale), which was estimated by elemental analysis to be a TFA salt with the formula $C_{19}H_{23}Cl_2NO_2 \cdot 0.85\,TFA \cdot 0.4\,H_2O$. ^{1}H NMR ([D$_6$]DMSO): 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 ppm (t, J=6.8 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 99.3%; HRMS (ESI+) calcd for $C_{19}H_{24}Cl_2NO_2$ 368.1179 [$M+H^+$, $^{35}Cl_2$], found 368.1162.

5-Chloro-2-[2-chloro-4-[(phenylamino)methyl]phenoxy]phenol (41b): The title compound was prepared by following 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 $NaHCO_3$. 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 (fs tt, J=7.3, 1.0 Hz, 1H), 6.24 (t, J=6.1 Hz, 1H), 4.21 ppm (d, J=6.1 Hz, 2H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 97.5%; HRMS (ESI+) calcd for $C_{19}H_{16}Cl_2NO_2$ 360.0553 [$M+H^+$, $^{35}Cl_2$], found 360.0557.

1-[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl]propan-1-one (42a): The title compound was prepared by following a published procedure.[53] The crude material was purified by flash chromatography on silica gel (hexanes, then hexanes/EtOAc 3:1–3:2) to provide the pure product as pale-red oil (259 mg, 80% on a 1.0 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (t, J=7.2 Hz, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 89.9%; MS (ESI+) *m/z* 325 [$M+H^+$, $^{35}Cl_2$].

[4-(2,4-Dichlorophenoxy)-3-methoxyphenyl](phenyl)methanone (42b): The title compound was prepared by following a published procedure.[53] The crude material was purified by flash chromatography on silica gel (hexanes, then hexanes/EtOAc 3:1–3:2) to provide the pure product as a pale-yellow oil (254 mg, 68% on a 1.0 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (s, 3H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 94.4%; MS (ESI+) *m/z* 373 [$M+H^+$, $^{35}Cl_2$].

1-[4-(2,4-Dichlorophenoxy)-3-hydroxyphenyl]propan-1-one (43a): The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 99.4%; HRMS (ESI−) calcd for $C_{14}H_{11}Cl_2O_3$ 309.0091 [$M-H]^+$, found 309.0091.

[4-(2,4-Dichlorophenoxy)-3-hydroxyphenyl](phenyl)methanone (43b): The title compound was prepared by following 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 mmol scale). ^{1}H NMR ([D$_6$]DMSO): 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 ppm (d, J=8.8 Hz, 1H); ^{13}C NMR ([D$_6$]DMSO): 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 ppm; HPLC purity 99.5%; MS (ESI+) *m/z* 359 [$M+H^+$, $^{35}Cl_2$]; HRMS (ESI+) calcd for $C_{19}H_{13}Cl_2O_3$ 359.0236 [$M+H^+$, $^{35}Cl_2$], found 359.0229.

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 greater than 70%; FAS II: type II fatty acid biosynthesis pathway; HFF: human foreskin fibroblasts; NADH: nicotinamide adenine dinucleotide; *Tg*ENR: *Toxoplasma gondii* enoyl-ACP reductase; TPSA: topological polar surface area; S_w: solubility in H_2O.

Acknowledgements

The authors are grateful for financial support provided by NIAID (U01 AI082180-01), the Rooney-Alden, Taub, Engel, Pritzker, Harris, Mussilami and Samuel families, and the Mann-Cornwell Family Foundation. Special thanks to Dr. W. Tückmantel for useful discussions and comments on the manuscript. S.P.M. is supported by an MRC Career Development fellowship (G1000567). We thank C. Van Tubbergen for her assistance with preparation of the manuscript.

Keywords: enoyl reductase · inhibitors · medicinal chemistry · *Toxoplasma gondii* · triclosan

[1] A. M. Tenter, A. R. Heckeroth, L. M. Weiss, *Int. J. Parasitol.* **2000**, *30*, 1217 – 1258.
[2] C. G. Lüder, W. Bohne, D. Soldati, *Trends Parasitol.* **2001**, *17*, 460 – 463.
[3] B. J. Luft, J. S. Remington, *Clin. Infect. Dis.* **1992**, *15*, 211 – 222.
[4] M. Hayde, A. Pollak in *Congenital Toxoplasmosis: Scientific Background, Clinical Management and Control* (Eds.: P. Ambroise-Thomas, E. Petersen), Springer, Amsterdam, **2000**; pp. 153 – 164.
[5] F. Roberts, M. B. Mets, D. J. Ferguson, R. O'Grady, C. O'Grady, P. Thulliez, A. P. Brézin, R. McLeod, *Arch. Ophthalmol.* **2001**, *119*, 51 – 58.
[6] J. P. Dubey, D. S. Lindsay, C. A. Speer, *Clin. Microbiol. Rev.* **1998**, *11*, 267 – 299.
[7] J. P. Dubey, *J. Eukaryotic Microbiol.* **2008**, *55*, 467 – 475.
[8] J. G. Montoya, O. Liesenfeld, *Lancet* **2004**, *363*, 1965 – 1976.
[9] G. I. McFadden, M. E. Reith, J. Munholland, N. Lang-Unnasch, *Nature* **1996**, *381*, 482.
[10] S. Köhler, C. F. Delwiche, P. W. Denny, L. G. Tilney, P. Webster, R. J. Wilson, J. D. Palmer, D. S. Roos, *Science* **1997**, *275*, 1485 – 1489.
[11] C. D. Goodman, G. I. McFadden, *Curr. Drug Targets* **2007**, *8*, 15 – 30.
[12] J. Wiesner, A. Reichenberg, S. Heinrich, M. Schlitzer, H. Jomaa, *Curr. Pharm. Des.* **2008**, *14*, 855 – 871.
[13] S. W. White, J. Zheng, Y. M. Zhang, C. O. Rock, *Annu. Rev. Biochem.* **2005**, *74*, 791 – 831.
[14] C. O. Rock, S. Jackowski, *Biochem. Biophys. Res. Commun.* **2002**, *292*, 1155 – 1166.
[15] S. Smith, *FASEB J.* **1994**, *8*, 1248 – 1259.
[16] R. J. Heath, C. O. Rock, *J. Biol. Chem.* **1995**, *270*, 26538 – 26542.
[17] R. J. Heath, N. Su, C. K. Murphy, C. O. Rock, *J. Biol. Chem.* **2000**, *275*, 40128 – 40133.
[18] R. P. Massengo-Tiasse, J. E. Cronan, *J. Biol. Chem.* **2008**, *283*, 1308 – 1316.
[19] H. Marrakchi, W. E. De Wolf, Jr., C. Quinn, J. West, B. J. Polizzi, C. Y. So, D. J. Holmes, S. L. Reed, R. J. Heath, D. J. Payne, C. O. Rock, N. G. Wallis, *Biochem. J.* **2003**, *370*, 1055 – 1062.
[20] K. Ryall, J. T. Harper, P. J. Keeling, *Gene* **2003**, *313*, 139 – 148.
[21] B. U. Samuel, B. Hearn, D. Mack, P. Wender, J. Rothbard, M. J. Kirisits, E. Mui, S. Wernimont, C. W. Roberts, S. P. Muench, D. W. Rice, S. T. Prigge, A. B. Law, R. McLeod, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14281 – 14286.
[22] D. J. Ferguson, F. L. Henriquez, M. J. Kirisits, S. P. Muench, S. T. Prigge, D. W. Rice, C. W. Roberts, R. L. McLeod, *Eukaryotic Cell* **2005**, *4*, 814 – 826.
[23] R. McLeod, S. P. Muench, J. B. Rafferty, D. E. Kyle, E. J. Mui, M. J. Kirisits, D. G. Mack, C. W. Roberts, B. U. Samuel, R. E. Lyons, M. Dorris, W. K. Milhous, D. W. Rice, *Int. J. Parasitol.* **2001**, *31*, 109 – 113.
[24] N. Surolia, A. Surolia, *Nat. Med.* **2001**, *7*, 167 – 173.
[25] E. S. Martins-Duarte, S. M. Jones, I. H. Gilbert, G. C. Atella, W. de Souza, R. C. Vommaro, *Parasitol. Int.* **2009**, *58*, 411 – 415.
[26] J. Z. Lu, S. P. Muench, M. Allary, S. Campbell, C. W. Roberts, E. Mui, R. L. McLeod, D. W. Rice, S. T. Prigge, *Parasitology* **2007**, *134*, 1949 – 1962.
[27] L. M. McMurry, M. Oethinger, S. B. Levy, *Nature* **1998**, *394*, 531 – 532.
[28] R. J. Heath, Y. T. Yu, M. A. Shapiro, E. Olson, C. O. Rock, *J. Biol. Chem.* **1998**, *273*, 30316 – 30320.
[29] W. H. Ward, G. A. Holdgate, S. Rowsell, E. G. McLean, R. A. Pauptit, E. Clayton, W. W. Nichols, J. G. Colls, C. A. Minshull, D. A. Jude, A. Mistry, D. Timms, R. Camble, N. J. Hales, C. J. Britton, I. Taylor, *Biochemistry* **1999**, *38*, 12514 – 12525.
[30] M. Kapoor, C. C. Reddy, M. V. Krishnasastry, N. Surolia, A. Surolia, *Biochem. J.* **2004**, *381*, 719 – 724.
[31] R. Perozzo, M. Kuo, A. S. Sidhu, J. T. Valiyaveettil, R. Bittman, W. R. J. Jacobs, D. A. Fidock, J. C. Sacchettini, *J. Biol. Chem.* **2002**, *277*, 13106 – 13114.
[32] S. Sivaraman, T. J. Sullivan, F. Johnson, P. Novichenok, G. Cui, C. Simmerling, P. J. Tonge, *J. Med. Chem.* **2004**, *47*, 509 – 518.
[33] J. S. Freundlich, J. W. Anderson, D. Sarantakis, H. M. Shieh, M. Yu, J. C. Valderramos, E. Lucumi, M. Kuo, W. R. J. Jacobs, D. A. Fidock, G. A. Schiehser, D. P. Jacobus, J. C. Sacchettini, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5247 – 5252.
[34] M. Chhibber, G. Kumar, P. Parasuraman, T. N. Ramya, N. Surolia, A. Surolia, *Bioorg. Med. Chem.* **2006**, *14*, 8086 – 8098.
[35] S. K. Tipparaju, D. C. Mulhearn, G. M. Klein, Y. Chen, S. Tapadar, M. H. Bishop, S. Yang, J. Chen, M. Ghassemi, B. D. Santarsiero, J. L. Cook, M. Johlfs, A. D. Mesecar, M. E. Johnson, A. P. Kozikowski, *ChemMedChem* **2008**, *3*, 1250 – 1268.
[36] J. S. Freundlich, F. Wang, C. Vilchèze, G. Gulten, R. Langley, G. A. Schiehser, D. P. Jacobus, W. R. J. Jacobs, J. C. Sacchettini, *ChemMedChem* **2009**, *4*, 241 – 248.
[37] S. K. Tipparaju, S. P. Muench, E. J. Mui, S. N. Ruzheinikov, J. Z. Lu, S. L. Hutson, M. J. Kirisits, S. T. Prigge, C. W. Roberts, F. L. Henriquez, A. P. Kozikowski, D. W. Rice, R. L. McLeod, *J. Med. Chem.* **2010**, *53*, 6287 – 6300.
[38] A. A. Divo, T. G. Geary, J. B. Jensen, *Antimicrob. Agents Chemother.* **1985**, *27*, 21 – 27.
[39] B. Wang, Y. Gua, G. Songa, T. Yang, L. Yang, J. Suob, *J. Mol. Catal. A* **2005**, *233*, 121 – 126.
[40] T. Goebel, N. Gauvry, P. Ducray (Novartis), WO2005/058802 A1, **2005**.
[41] R. Sterzycki, *Synthesis* **1979**, 724 – 725.
[42] Z. Lu, C. Hu, J. Guo, J. Li, Y. Cui, Y. Jia, *Org. Lett.* **2010**, *12*, 480 – 483.
[43] T. W. Baughman, J. C. Sworen, K. B. Wagener, *Tetrahedron* **2004**, *60*, 10943 – 10948.
[44] F. Shi, J. P. Waldo, Y. Chen, R. C. Larock, *Org. Lett.* **2008**, *10*, 2409 – 2412.
[45] F. Himo, T. Lovell, R. Hilgraf, V. V. Rostovtsev, L. Noodleman, K. B. Sharpless, V. V. Fokin, *J. Am. Chem. Soc.* **2005**, *127*, 210 – 216.
[46] B. N. Naidu, J. Banville, F. Beaulieu, T. P. Connolly, M. R. Krystal, J. D. Matiskella, C. Ouellet, S. Plamondon, R. Remillard, M. E. Sorenson, Y. Ueda, M. A. Walker (Bristol-Myers Squibb), WO2007/064316 A1, **2007**.
[47] P. R. Brooks, M. C. Wirtz, M. G. Vetelino, D. M. Rescek, G. F. Woodworth, B. P. Morgan, J. W. Coe, *J. Org. Chem.* **1999**, *64*, 9719 – 9721.
[48] Y. Xia, K. Cao, Y. Zhou, M. R. Alley, F. Rock, M. Mohan, M. Meewan, S. J. Baker, S. Lux, C. Z. Ding, G. Jia, M. Kully, J. J. Plattner, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2533 – 2536.
[49] G. Charalambidis, K. Ladomenou, B. Boitrel, A. G. Coutsolelos, *Eur. J. Org. Chem.* **2009**, 1263 – 1268.
[50] S. Wittich, H. Scherf, C. Xie, G. Brosch, P. Loidl, C. Gerhäuser, M. Jung, *J. Med. Chem.* **2002**, *45*, 3296 – 3309.
[51] C. W. Liskey, X. Liao, J. F. Hartwig, *J. Am. Chem. Soc.* **2010**, *132*, 11389 – 11391.

[52] V. Ehmke, C. Heindl, M. Rottmann, C. Freymond, W. B. Schweizer, R. Brun, A. Stich, T. Schirmeister, F. Diederich, *ChemMedChem* **2011**, *6*, 273 – 278.
[53] J. C. Barrow, K. E. Rittle, T. S. Reger, Z. Q. Yang, P. Bondiskey, G. B. McGaughey, M. G. Bock, G. D. Hartman, C. Tang, J. Ballard, Y. Kuo, T. Prueksaritanont, C. E. Nuss, S. M. Doran, S. V. Fox, S. L. Garson, R. L. Kraus, Y. Li, M. J. Marino, V. K. Graufelds, V. N. Uebele, J. J. Renger, *ACS Med. Chem. Lett.* **2010**, *1*, 75 – 79.
[54] S. P. Muench, S. T. Prigge, R. McLeod, J. B. Rafferty, M. J. Kirisits, C. W. Roberts, E. J. Mui, D. W. Rice, *Acta Crystallogr. Sect. D* **2007**, *63*, 328 – 338.
[55] E. M. L. Cohen, K. S. Machado, M. Cohen, O. N. de Souza, *BMC Genomics* **2011**, *12*, S7.
[56] M. J. Soltis, H. J. Yeh, K. A. Cole, N. Whittaker, R. P. Wersto, E. C. Kohn, *Drug Metab. Dispos.* **1996**, *24*, 799 – 806.
[57] S. P. Muench, S. T. Prigge, L. Zhu, M. J. Kirisits, C. W. Roberts, S. Wernimont, R. McLeod, D. W. Rice, *Acta Crystallogr. Sect. F* **2006**, *62*, 604 – 606.
[58] J. H. Zhang, T. D. Chung, K. R. Oldenburg, *J. Biomol. Screening* **1999**, *4*, 67 – 73.
[59] E. J. Mui, D. Jacobus, W. K. Milhous, G. Schiehser, H. Hsu, C. W. Roberts, M. J. Kirisits, R. McLeod, *Antimicrob. Agents Chemother.* **2005**, *49*, 3463 – 3467.
[60] D. G. Mack, R. McLeod, *Antimicrob. Agents Chemother.* **1984**, *26*, 26 – 30.
[61] R. McLeod, D. Mack, R. Foss, K. Boyer, S. Withers, S. Levin, J. Hubbell, *Antimicrob. Agents Chemother.* **1992**, *36*, 1040 – 1048.
[62] E. Holfels, J. McAuley, D. Mack, W. K. Milhous, R. McLeod, *Antimicrob. Agents Chemother.* **1994**, *38*, 1392 – 1396.
[63] F. Roberts, C. W. Roberts, J. J. Johnson, D. E. Kyle, T. Krell, J. R. Coggins, G. H. Coombs, W. K. Milhous, S. Tzipori, D. J. Ferguson, D. Chakrabarti, R. McLeod, *Nature* **1998**, *393*, 801 – 805.
[64] R. Estes, N. Vogel, D. Mack, R. McLeod, *Antimicrob. Agents Chemother.* **1998**, *42*, 2036 – 2040.
[65] E. Zuther, J. J. Johnson, R. Haselkorn, R. McLeod, P. Gornicki, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 13387 – 13392.
[66] M. J. Kirisits, E. Mui, R. McLeod, *Int. J. Parasitol.* **2000**, *30*, 149 – 155.
[67] C. W. Roberts, F. Roberts, F. L. Henriquez, D. Akiyoshi, B. U. Samuel, T. A. Richards, W. Milhous, D. Kyle, L. McIntosh, G. C. Hill, M. L. Chaudhuri, S. Tzipori, R. McLeod, *Int. J. Parasitol.* **2004**, *34*, 297 – 308.
[68] E. J. Mui, G. A. Schiehser, W. K. Milhous, H. Hsu, C. W. Roberts, M. Kirisits, S. Muench, D. Rice, J. P. Dubey, J. W. Fowble, P. K. Rathod, S. F. Queener, S. R. Liu, D. P. Jacobus, R. McLeod, *PLoS Neglected Trop. Dis.* **2008**, *2*, e190.
[69] A. Fomovska, Q. Q. Huang, K. El Bissati, E. J. Mui, W. H. Witola, G. Cheng, Y. Zhou, C. Sommerville, C. W. Roberts, S. Bettis, S. T. Prigge, G. A. Afanador, M. R. Hickman, P. J. Lee, S. E. Leed, J. M. Auschwitz, M. Pieroni, J. Stec, S. P. Muench, D. W. Rice, A. P. Kozikowski, R. McLeod, *Antimicrob. Agents Chemother.* **2012**, *56*, 2666 – 2682.
[70] B.-S. Lai, W. Witola, K. El Bissati, E. Mui, A. Fomovska, R. McLeod, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 14182 – 14187.
[71] A. Fomovska, R. D. Wood, E. Mui, J. P. Dubey, L. R. Ferriera, M. Hickman, P. J. Lee, S. E. Leed, J. M. Auschwitz, W. J. Welsh, C. Sommerville, S. Woods, C. W. Roberts, R. McLeod, *J. Med. Chem.* **2012**, *55*, 8375 – 8391.
[72] J. D. Johnson, R. A. Dennull, L. Gerena, M. Lopez-Sanchez, N. E. Roncal, N. C. Waters, *Antimicrob. Agents Chemother.* **2007**, *51*, 1926 – 1933.
[73] D. Plouffe, A. Brinker, C. H. McNamara, N. Kato, K. Kuhen, A. Nagle, F. Adrian, J. T. Matzen, P. Anderson, T. Nam, N. S. Gray, A. Chatterjee, J. Janes, S. F. Yan, R. Trager, J. S. Caldwell, P. G. Schultz, Y. Zhou, E. A. Winzeler, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9059 – 9064.
[74] M. Yu, T. R. Kumar, L. J. Nkrumah, A. Coppi, S. Retzlaff, C. D. Li, B. J. Kelly, P. A. Moura, V. Lakshmanan, J. S. Freundlich, J. C. Valderramos, C. Vilcheze, M. Siedner, J. H. Tsai, B. Falkard, A. B. Sidhu, L. A. Purcell, P. Gratraud, L. Kremer, A. P. Waters, G. Schiehser, D. P. Jacobus, C. J. Janse, A. Ager, W. R. Jacobs, Jr., J. C. Sacchettini, V. Heussler, P. Sinnis, D. A. Fidock, *Cell Host Microbe* **2008**, *4*, 567 – 78.
[75] A. M. Vaughan, M. T. O'Neill, A. S. Tarun, N. Camargo, T. M. Phuong, A. S. Aly, A. F. Cowman, S. H. Kappe, *Cell. Microbiol.* **2009**, *11*, 506 – 520.
[76] G. Cheng, S. P. Muench, Y. Zhou, G. A. Afanador, E. J. Mui, A. Fomovska, B. S. Lai, S. T. Prigge, S. Woods, C. W. Roberts, M. R. Hickman, P. J. Lee, S. E. Leed, J. M. Auschwitz, D. W. Rice, R. McLeod, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2035 – 2043.
[77] S. P. Muench, J. Stec, Y. Zhou, G. A. Afanador, M. J. McPhillie, M. R. Hickman, P. J. Lee, S. E. Leed, J. M. Auschwitz, S. T. Prigge, D. W. Rice, R. McLeod, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3551 – 3555.

Received: February 1, 2013
Revised: April 26, 2013
Published online on June 14, 2013