



This is a repository copy of *Design, synthesis, and biological activity of diaryl ether inhibitors of Toxoplasma gondii enoyl reductase*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/110841/>

Version: Accepted Version

Article:

Cheng, G., Muench, S.P., Zhou, Y. et al. (13 more authors) (2013) Design, synthesis, and biological activity of diaryl ether inhibitors of Toxoplasma gondii enoyl reductase. *Bioorganic and Medicinal Chemistry Letters*, 23 (7). pp. 2035-2043. ISSN 0960-894X

<https://doi.org/10.1016/j.bmcl.2013.02.019>

Article available under the terms of the CC-BY-NC-ND licence
(<https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Reuse

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: <https://creativecommons.org/licenses/>

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

Published in final edited form as:

Bioorg Med Chem Lett. 2013 April 1; 23(7): 2035–2043. doi:10.1016/j.bmcl.2013.02.019.

Design, synthesis, and biological activity of diaryl ether inhibitors of *Toxoplasma gondii* enoyl reductase

Gang Cheng^{a,*}, Stephen P. Muench^{b,*}, Ying Zhou^{c,*}, Gustavo A. Afanador^{d,*}, Ernest J. Mui^c, Alina Fomovska^c, Bo Shiun Lai^c, Sean T. Prigge^d, Stuart Woods^e, Craig W. Roberts^e, Mark R. Hickman^g, Patty J. Lee^g, Susan E. Leed^g, Jennifer M. Auschwitz^g, David W. Rice^f, and Rima McLeod^{c,*}

^aDrug Discovery Program, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois, 60612, United States

^bSchool of Biomedical Sciences, University of Leeds, LS2 9JT, Leeds

^cDepartment 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, Illinois 60637, United States

^dJohns Hopkins School of Public Health Rm. E5132, 615 N. Wolfe St. Baltimore MD 21205, United States

^eStrathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, U.K.

^fDepartment of Molecular Biology and Biotechnology, The University of Sheffield, Sheffield, U.K.

^gDepartment of Discovery, Division of Experimental Therapeutics Walter Reed Army Institute of Research, Rm 2N61 503 Robert Grant Avenue, Silver Spring, MD 20910

Abstract

Triclosan is a potent inhibitor of *Toxoplasma gondii* enoyl reductase (TgENR), which is an essential enzyme for parasite survival. In view of triclosan's poor druggability, which limits its therapeutic use, a new set of B-ring modified analogs were designed to optimize its physico-chemical properties. These derivatives were synthesized and evaluated by in vitro assay and TgENR enzyme assay. Some analogs display improved solubility, permeability and a comparable MIC₅₀ value to that of triclosan. Modeling of these inhibitors revealed the same overall binding mode with the enzyme as triclosan, but the B-ring modifications have additional interactions with the strongly conserved Asn130.

© 2013 Elsevier Ltd. All rights reserved.

*To whom correspondence should be addressed. Phone: 773-834-4152. Fax: 773-834-3577. rmcLeod@midway.uchicago.edu.

*These authors contributed equally

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary Material

Supplementary data (experimental procedures and spectral data) associated with this article can be found, in the online version.

Keywords

TgENR; Triclosan; ADMET; Toxoplasma

Toxoplasma gondii (*T. gondii*) is an obligate intracellular, protozoan parasite belonging to the phylum Apicomplexa. It infects over one third of the world's population. It can cause substantial morbidity and mortality.¹⁻⁶ People are mainly infected by unrecognized and inadvertent ingestion of materials contaminated with oocysts from the feces of cats^{4, 5} or eating undercooked meat containing bradyzoite-stage parasites^{6, 7}. Generally most infections in healthy individuals are asymptomatic and self-limiting. However, in immunocompromised persons, *T. gondii* infection can cause eye and brain disease such as toxoplasmic encephalitis, chorioretinitis, and death.^{8, 9} Infection acquired during pregnancy can be especially severe; it can transmit from mother to the fetus, leading to congenital toxoplasmosis which may result in abortion, neonatal death, or fetal abnormalities.^{2, 10-16} Currently, there is no vaccine available to prevent human infection from this pathogen. Antifolate agents, sulfadiazine and pyrimethamine, are two primary medicines for treatment of *T. gondii* infection in humans.^{2, 15} Although these medicines are effective against tachyzoites in the acute stage of the disease, they do not eradicate encysted, latent bradyzoites. Furthermore, these therapies can be associated with side effects such as bone marrow depression, hypersensitivity and skin rashes.^{15, 16} There is an urgent need to develop new anti-*T. gondii* medicines that are both efficacious and nontoxic to humans.

One attractive target for chemotherapeutic intervention against apicomplexan parasites is the prokaryotic-like type II fatty acid biosynthesis (FAS-II) pathway.¹⁷⁻²¹ In *T. gondii*, this apicoplast-localized FAS-II pathway has been proved to be essential for parasite survival by both *in vitro* and *in vivo* studies.²¹ Type II FAS is fundamentally divergent from the analogous FAS I pathway in mammals. In eukaryotes, fatty acid biosynthetic enzymes integrate on a single multifunctional polypeptide (FASI), whereas fatty acid synthesis in prokaryotes utilizes a set of distinct enzymes composing the FAS-II pathway.²² Fatty acid biosynthesis is an iterative process beginning with condensation of acetyl-Coenzyme A (acetyl-CoA) with a growing fatty acid chain. In *T. gondii*, enoyl-acyl carrier protein (ACP) reductase (ENR) is responsible for the final reductive step in each round of the fatty acid chain elongation, the NADH-dependent reduction of trans-2-enoyl-ACP to acyl-ACP.²³ There are a number of known bacterial and parasitic ENR inhibitors such as diazaborines, isoniazid and as well as triclosan which is a slow, tight-binding inhibitor of ENR.²⁴⁻²⁶ Moreover, triclosan inhibits TgENR enzymatic activity with an IC₅₀ value of less than 20 nM²⁸ and inhibits the growth of *T. gondii* parasites with an IC₅₀ value of ~200nM.¹⁸

Although triclosan is a potent inhibitor of TgENR, the diphenyl ether has low water solubility and a high ClogP value. Another major challenge for the development of medicines against targets which reside within the apicoplast of apicomplexan parasites is the need for the inhibitors to cross the four membranes of the parasite-specific organelle, in addition to the barriers set by both host cell and the parasite.²⁷ In order to overcome these structural drawbacks and address the uptake problem, structure-based modification of triclosan was directed by improving the ADMET (absorption, distribution, metabolism, excretion and toxicity) profiles with special focus on the increase of aqueous solubility and permeability. We have previously shown that the A-ring of triclosan can be modified to exploit an additional space at the base of the inhibitor binding pocket.²⁸ Furthermore, we have shown that substitution on the B-ring may also be tolerated to produce effective triclosan analogues {Stec et al., *in preparation*}. This study has now been extended to show that modifications of the B-ring can exploit the channel which leads from the substrate binding pocket to the outside solvent. We report herein on our efforts in developing novel

4'-substituted and B-ring substituted triclosan analogs with better physico-chemical properties.

Triclosan analogs substituted at the 4' position were generated from the commercially available phenol **1** using the methods outlined in Scheme 1.

The naphthyl phenyl ether derivative **2** was synthesized by Cu-promoted Chan-Lam coupling²⁹ of the phenol **1** with the 2-naphthyl boronic acid and subsequent O-demethylation. Compound **3** with a trifluoromethyl substituent at the 4' position was obtained from phenol **1** and 2-chloro-1-fluoro-4-(trifluoromethyl) benzene via nucleophilic substitution and demethylation. Cu-promoted Chan-Lam coupling²⁹ of the phenol **1** with the 4-bromophenylboronic acid and 4-formylphenylboronic acid gave the corresponding intermediate bromide **4a** and aldehyde **4b**, respectively. Palladium-catalyzed amination³⁰ of the bromoarene **4a** and reductive amination of the benzaldehyde **4b** afforded, after deprotection, the piperazine or morpholine inhibitors **5a-d**. The 4'-sulfonamide derivatives **7a, b** were prepared by Cu-catalyzed arylation of phenol **1** with 4-iodobenzenesulfonamides³¹ and subsequent demethylation. Compound **10** was prepared by coupling of 2-amino-5-chlorophenol **8** with 1-methyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid followed by demethylation. Similar to the procedure for **7a**, Compound **19** was obtained by treatment of phenol **1** with 6-bromoquinoline and subsequent demethylation. The furyl phenyl ether analogs (**13, 14a-c**) were obtained in 2–4 steps utilizing the synthetic routes shown in Scheme 2. Reaction of phenol **1** with 5-nitrofuran-2-carbaldehyde provided the furyl phenyl ether aldehyde **11**.³² This intermediate was subjected to NaClO₂ oxidation and BBr₃ mediated demethylation to afford the furan-2-carboxylic acid **13**. Next, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI), 1-hydroxybenzotriazole (HOBt) and *N, N*-diisopropylethylamine (DIEA) carboxylic acid **13** was coupled with three different amines to generate the desired ligands **14a-c**.

Compounds **17a-c** were prepared in an analogous manner as described for **14a**, starting from **1** via a sequence of steps involving nucleophilic aromatic substitution, oxidation, demethylation and amide bond formation (Scheme 3). Compound **18** was obtained from **15** through reductive amination and subsequent demethylation.

Compounds were tested against TgENR, *T. gondi* *in vitro* and *in vivo*, and *P. falciparum* *in vitro* using methods that have been described in the recent literature^{28, 33} For comparison of parasite burden between treatment groups, analysis of variance (ANOVA) was performed with group and run as factors. Due to evidence of non-normality, natural log-transformed parasite burden was used in the analysis. (Also, see Supplementary Material).

The crystal structure of TgENR in complex with NAD⁺ and triclosan,³⁴ showed that the 4-chloro phenoxy ring (A-ring) of triclosan participates in a π - π stacking interaction with NAD⁺, and a hydrogen bond forms between the hydroxyl group and Tyr189. However, the 2,4-dichlorophenoxy ring (B-ring) engages only in van der Waals interactions within a pocket encompassed by the peptide backbone of residues Leu128 to Ala131, the pyrophosphate and nicotinamide moieties of NAD⁺, and the side chains of Val134, Met193, Ala231 and Ile235. Moreover, there remains some additional space around the B-ring that could be exploited. In particular the B-ring is exposed to the outside solvent via a channel which would allow the fatty acyl substrate attached to the acyl carrier protein to enter the active site (Fig. 1A).

In view of the space around the B-ring which could be exploited, we devised a modification strategy to change the B- ring, incorporating additional polar groups to optimize the physicochemical properties (such as permeability and solubility) while keeping the A-ring

intact. These modifications were guided by computer-aided ADMET prediction (ADMET suite from ACD/labs). In total, 19 triclosan analogs have been designed with either substitutions at the 4'-position of the B-ring or with the complete replacement of its benzene ring by heteroaromatic groups. The biological test data are shown in Table 1 along with some calculated ADMET properties.

Modeling studies indicated that these inhibitors should have similar binding positions as triclosan with respect to the A-ring. While the changes on the B-ring in these derivatives might protrude out from the triclosan binding pocket using the natural channel which the fatty acid substrate uses when attached to the acyl carrier protein (Fig 1A). An attractive additional interaction which these substituted inhibitors may acquire is with Asn130 (TgENR numbering) which is fully conserved within the apicomplexan family (Fig 1A, 1B). The inhibitory properties and cytotoxicity data of the tested inhibitors are shown in Table 1.

Compounds 2, 3 and 10 and 19

Initially, the B-ring of triclosan was substituted with a bulkier naphthalene ring system (Compound **2**) to explore the size of the pocket around the B-ring. This had the adverse effect of making the inhibitor even less soluble than triclosan itself, which is unpractical for a therapeutic drug. Despite the good activity of this compound, it was therefore not considered worthwhile to develop this family of compounds further. This result did however, confirm that the B-ring of triclosan can accommodate significant change and still be an effective ENR inhibitor. To increase the solubility, we prepared compound **19** in which the naphthalene ring was replaced by a quinoline ring. However, it lost some enzyme activity. In order to investigate the role of the oxygen atom in the ether linker and to further understand the role of the B-ring, we prepared compound **10**. Despite the A-ring system being intact, compound **10** was relatively inactive compared to triclosan and thus further modifications of this compound were not attempted. The low activity of compound **10** is primarily due to the loss of the bridging oxygen which has been shown to play an important role in triclosan's efficacy³⁵. Excellent activity was observed for compound **3** in which the chlorine atom attached to the C4 of triclosan was replaced by a trifluoromethyl group. The comparable activity of this derivative to triclosan is not surprising due to the close similarity in size between the trifluoromethyl and chloro substituent. Since compound **3** has similar physico-chemical properties to triclosan, and therefore is thought to share many of the known problems, we did not further optimize this compound. Instead, we exploited the fact that we were able to substitute the 4-chlorine atom of the B-ring without significant loss of efficacy, and we made a series of substitutions at this position of the triclosan B-ring.

Piperazine and morpholine based inhibitors 5a-d

One of the most promising compound families developed involved the replacement of chlorine at the C4-position of the B-ring with a piperazine moiety (**5a** and **5c**). Incorporation of this polar group would greatly improve the water solubility according to the ADMET prediction (360 mg/L for **5a** and 160 mg/L for **5c** versus 4.6 mg/L for triclosan). Furthermore, placing an acetyl group onto the N4 position of the piperazine derivative **5c** improved its inhibitory properties in the enzyme assay (81% inhibition at 1 μ M for **5c** vs. 25% for **5a**) (Table 1). However, this increased potency is offset by a slight difference in solubility between **5a** and **5c**. This highlights the fine balance between the inhibitory properties and other important features such as solubility and cellular permeability. Modeling of these two compounds using the TgENR/NAD⁺/triclosan complex crystal structure³⁴ as a guide indicated that these compounds should be able to form a hydrogen bond between the N4 of the piperazine ring and the oxygen atom of the Asn130 side chain (Figure 2).

The replacement of N4 with oxygen (**5b**) results in a significant decrease in efficacy (Table 1). This is likely due to the loss of a hydrogen bond to the O4 atom through the subtle differences in a morpholine ring structure when compared to a piperazine structure. Insertion of a flexible carbon linker between the piperazine group and the B-ring (**5d**) resulted in a significant loss of potency when compared to **5a**. Since the only difference between these inhibitors is the methylene linker, the reduction in potency can be explained by the shift of the piperazine group further out of the binding pocket so that it can no longer maintain a hydrogen bond with Asn130. These results show that the N4 position of the rigidly attached piperazine group is important for good inhibitor activity and that replacement of the chlorine atom at the C4'-position on the B-ring of triclosan by a large piperazine group yields a scaffold suited for further development of TgENR inhibitors.

Benzenesulfonamide based inhibitors (7a and 7b)

Two compounds were designed that feature a replacement of the chlorine atom at the C4-position on the B-ring of triclosan by a pyrrolidinylsulfonyl (**7a**) and piperidinylsulfonyl group (**7b**). These inhibitors displayed similar activities in both the enzyme-based and cell-based assays (Table 1) but were significantly less active than compounds **5b** and **5c**. We concluded that the observed reduction in potency is unlikely to be an effect of their piperidine or pyrrole ring motifs but instead an effect of their shared sulfonyl motifs suggesting that this is an unsuitable substitution. The modeling of these inhibitors showed no steric clashes within the binding pocket which would cause a decrease in binding affinity. However, the sulfonyl groups would be able to form a hydrogen bond with the main chain nitrogen of Asn130 and Gly131. This may inadvertently cause a shift of the B-ring moiety which has a secondary effect on the A-ring such that it no longer has optimum stacking interactions with the NAD⁺ cofactor. Further crystallographic studies would be required to test this hypothesis.

Furan ring based inhibitors (13 and 14a-c)

The next set of compounds was designed with a 2-furyl group to replace the B-ring of triclosan. The furan ring was then further substituted at C5 with several chemical motifs. Attachment of an *N*-acetylpiperazine ring through a carboxyl group (**14c**) would greatly increase the compound's solubility to 1600mg/L according to the calculated ADMET prediction. However, a significant loss of potency was observed when **14c** was tested in the TgENR assay. A second approach was to attach a carboxyl group (**13**) or related amide group (**14a**) to the furan ring at its C5-position. These inhibitors showed similar results in the parasite tissue culture assay and shared similar calculated solubility values. However, they varied in their ability to inhibit TgENR (compound **13** displayed 79% inhibition at 1 μ M whereas compound **14a** displayed only 46% inhibition). The improved inhibitory activity of compound **13** is likely to be due to the carboxyl group. However, the best compound of this family, **14b**, contains the amide motif at this position with an additional isoxazole group. We conclude that either a carboxyl or amide group is tolerated at this position, with the substituent on the amide nitrogen playing an important role in inhibitor efficacy, as an amide group not bearing additional functionality is not sufficient to improve inhibitor binding. In order to further understand the possible role that the additional isoxazolyl group at the amine nitrogen might play, this inhibitor was modeled into the TgENR/NAD⁺/triclosan complex crystal structure. The modeling result showed that as with the piperazine based inhibitors (**5a**, **5c**), Asn130 may play an important role in inhibitor binding by forming a hydrogen bond to the inhibitor. Inhibitor **14b** also showed a greatly improved solubility (210 mg/L) when compared to triclosan (4.6 mg/L) without a significant effect on its predicted cellular permeability properties. This compound family has shown that not just substituents added to the B-ring of triclosan are tolerated but that the B-ring

itself can be replaced without significant loss of potency. This is an important insight when trying to avoid some of the major downsides of triclosan such as low solubility, stability.

Thiophene ring based inhibitors (16, 17a-c, 18)

Since triclosan can accommodate the replacement of the B-ring with a 2-furyl group, our next step in designing novel ENR inhibitors was to replace the B-ring with a 2-thienyl group, to which a variety of chemically distinct groups were attached at its C5-position. The smallest group that we attached to the thiophene ring was carboxyl (**16**), which did not produce an effective inhibitor like the similar compound **13**. This is likely the case because no additional inhibitor–enzyme interactions can be formed to the small carboxyl group. The most promising of the thiophene-based inhibitors is compound **18** which showed a good inhibitory effect in the parasite tissue cell culture assay and moderate activity in the enzyme assay, coupled with a significant improvement in solubility compared to triclosan. Modeling of compound **18**, which contains a 4-acetylpiperazin-1-yl group attached to the thiophene ring through a methylene linker, showed again the importance of Asn130 on the efficacy of these inhibitors as it may hydrogen bond to the piperazine moiety (Table 1, Fig. 2). Interestingly, **14c** is similar to **18** in that it also contains a N-acetylated piperazine ring at its terminal. However, this group is attached through a furan ring and carbonyl group in compound **14c** rather than a thiophene ring and methylene group in compound **18**. Since the furan and thiophene ring systems are similar, it is conceivable that the flexible methylene linker permits the piperazine ring in **18** to adopt a favorable position with respect to hydrogen bonding with Asn130 while the rigid carbonyl linker in **14c** does not. The carbonyl linker in the effective furan-based compound **14b** does not have this negative effect due to the different orientation of the hydrogen bond acceptors in its isoxazolyl group compared to the N4-atom of the piperazinyl group in compound **18**. Moreover, compounds **17a**, **17b**, and **17c**, which contain a carbonyl rather than methylene linker between their B rings and terminal amine moieties, also exhibit significant decreases in activity.

In vivo experiments

Compound **5c** was selected for further biological evaluation in a *T. gondii* murine infection model (Figure 3). In 3 separate, replicate experiments mice treated with compound **5c** had fewer parasites on average when compared with the DMSO control on the 6th day after infection. Although parasite number varied between individual mice, these modest differences reached significance ($p < 0.05$), in two of the three replicate experiments. These data are shown pooled in Figure 3 with a separate symbol for each experiment. When the data were pooled the difference between the amount of parasite burden in DMSO control and compound **5c** treated mice was significant. The p-value for the treatment group comparison was 0.003. A lower dose of 50mg/kg was not effective and a higher dose of 100mg/kg was not completely soluble. The compound was toxic at 75mg/kg by the seventh day after infection. Thus, although the predicted ADMET properties were superior to those of triclosan, this compound did not prove to be superior in these *in vivo* tests where triclosan is active at 25mg/kg. The relatively lower efficacy may be due to metabolic instability of compound **5c**.

Activity against *Plasmodium falciparum*

Compound **5c** was also tested against *P. falciparum* strain D6 (CDC/Sierra Leone) and TM91C235 (WRAIR, Thailand). Results are in Table 2.

Compound **5c** was inactive against the chloroquine sensitive D6 and the chloroquine resistant C235 strains. Modeling of **5c** within the PfENR active site shows that there is one

subtle difference which may affect 5c binding whereby ASN which H-bonds to the inhibitor is followed by Gly-Pro in TgENR and Ala-Lys in PfENR. This change alters the main chain position about the exit of 5c such that it makes less favourable packing interactions. However, it is unlikely that this small change would abolish activity and it is more likely that this can be explained by the non-essential nature of ENR within *P. falciparum* during its blood stage development³⁷. A crystal structure would be needed to determine if that prediction is correct and explains the lack of activity of 5c for *P. falciparum*. Since the fatty acid biosynthesis enzyme ENR plays a key role in the development of liver stage malarial parasites, it also will be of interest in the future to learn whether the new inhibitors that utilize the space in the enzyme that accepts B ring modifications would improve the activity of such inhibitors against the liver stage organisms.

The use of triclosan-based compounds as possible inhibitors of apicomplexan parasites has become an intense area of study since the parasitic ENR was validated as a novel target.^{17, 18} However, despite its potency, triclosan displays a range of poor ADMET properties. In particular, it is very poorly soluble in water. In order to address some of these problems we synthesized a range of inhibitors bearing modifications to the B-ring of the triclosan scaffold. Compounds **2** and **3** showed that, as previously reported,³⁶ the B-ring of triclosan can be modified without a significant reduction in inhibitor efficacy. The structural changes that were explored were based on chemical units comprised of piperazine, furan and thiophene ring systems. These added chemical groups were designed to occupy the channel which leads from the triclosan binding site to the area exposed to outside solvent; this area is thought to be the portal through which insertion of fatty acid substrates by the ACP takes place.

Because of the important role that this portal appears to play, as it is lined by relatively well conserved amino acid residues, the use of this region in the design of new therapeutics appears justified. In particular, the strongly conserved amino acid residue Asn130 (Fig. 1A) may play an important role in binding a number of these ligands. For those compounds which are incapable of forming an H-bond interaction with Asn130, only poor inhibitory activity is observed. Of the compounds disclosed herein, three compounds, namely **14b**, **5c**, and **18** may serve as leads candidates for further rounds of SAR optimization, as these compounds appear to show drug-like properties along with the lack of toxicity to human fibroblasts in comparison to their toxicity to the parasite.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful for the financial support provided by NIAID grant U01 AI082180-0101 and the Wellcome Trust and the support of the Mann and Cornwell, Rooney-Alden, Taub, Engel and Mussilami families. SPM is funded by an MRC Career Development Fellowship (G1000567). The authors also thank Alan Kozikowski for his contributions. They also thank K. Wroblewski for her assistance with statistical analyses of the *in vivo* data. The opinions of the authors from Walter Reed Army Institute of Research are their own and do not reflect the official views of the Department of Defense.

References

1. Hill D, Dubey JP. Clin. Microbiol. Infect. 2002; 8:634. [PubMed: 12390281]
2. McLeod R, Boyer K, Karrison T, Kasza K, Swisher C, Roizen N, Jalbrzikowski J, Remington J, Heydemann P, Noble AG, Mets M, Holfels E, Withers S, Latkany P, Meier P. Clin. Infect. Dis. 2006; 42:1383. [PubMed: 16619149]

3. Boyer, K.; Marcinak, J.; McLeod, R. Principles and Practice of Pediatric Infectious Diseases. 4th Ed.. Long, S.; Pickering, LK.; Prober, CG., editors. Churchill Livingstone, New York: 2009. section 274
4. Hill D, Coss C, Dubey JP, Wroblewski K, Sautter M, Hosten T, Munoz-Zanzi C, Mui E, Withers S, Boyer K, Hermes G, Coyne J, Jagdis F, Burnett A, McLeod P, Morton H, Robinson D, McLeod R. J. Parasitol. 2011; 97:328. [PubMed: 21506817]
5. Boyer K, Hill D, Mui E, Wroblewski K, Karrison T, Dubey JP, Sautter M, Noble AG, Withers S, Swisher C, Heydemann P, Hosten T, Babiarz J, Lee D, Meier P, McLeod R. Clin. Infect. Dis. 2011; 53:1081. [PubMed: 22021924]
6. Desmonts G, Couvreur J. N. Engl. J. Med. 1974; 290:1110. [PubMed: 4821174]
7. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. Emerg. Infect. Dis. 1999; 5:607. [PubMed: 10511517]
8. Luft BJ, Remington JS. Clin. Infect. Dis. 1992; 15:211. [PubMed: 1520757]
9. Porter SB, Sande MA. N. Engl. J. Med. 1992; 327:1643. [PubMed: 1359410]
10. Swisher CN, Boyer K, McLeod R. Semin. Pediatr. Neurol. 1994; 1:4. [PubMed: 9422215]
11. Olariu TR, Remington JS, McLeod R, Alam A, Montoya JG. Pediatr. Infect. Dis. J. 2011; 30:1056. [PubMed: 21956696]
12. McLeod R, Mack DG, Boyer K, Mets M, Roizen N, Swisher C, Patel D, Beckmann E, Vitullo D, Johnson D, et al. J. Lab. Clin. Med. 1990; 116:623. [PubMed: 2146348]
13. McGee T, Wolters C, Stein L, Kraus N, Johnson D, Boyer K, Mets M, Roizen N, Beckman J, Meier P, et al. Otolaryngol. Head Neck Surg. 1992; 106:75. [PubMed: 1734373]
14. McAuley J, Boyer KM, Patel D, Mets M, Swisher C, Roizen N, Wolters C, Stein L, Stein M, Schey W, et al. Clin. Infect. Dis. 1994; 18:38. [PubMed: 8054436]
15. Remington, JS.; McLeod, R.; Thulliez, P.; Desmonts, G. Toxoplasmosis. In: Remington, J.; Klein, J., editors. Infectious Diseases of the Fetus and Newborn Infant. 7th Ed.. Philadelphia, WB: Saunders; 2011.
16. McLeod R, Khan AR, Noble GA, Latkany P, Jalbrzikowski J, Boyer K. Pediatr. Infect. Dis. J. 2006; 25:270. [PubMed: 16511396]
17. Zuther E, Johnson JJ, Haselkorn R, McLeod R, Gornicki P. Proc. Natl. Acad. Sci. USA. 1999; 96:13387. [PubMed: 10557330]
18. McLeod R, Muench SP, Rafferty JB, Kyle DE, Mui EJ, Kirisits MJ, Mack DG, Roberts CW, Samuel BU, Lyons RE, Dorris M, Milhous WK, Rice DW. Int. J. Parasitol. 2001; 31:109. [PubMed: 11239932]
19. Ben Mamoun C, Prigge ST, Vial H. Drug Dev. Res. 2010; 71:44. [PubMed: 20559451]
20. Lu JZ, Muench SP, Allary M, Campbell S, Roberts CW, Mui E, McLeod RL, Rice DW, Prigge ST. Parasitology. 2007; 134:1949. [PubMed: 17697396]
21. Mazumdar J, E HW, Masek K, C AH, Striepen B. Proc. Natl. Acad. Sci. USA. 2006; 103:13192. [PubMed: 16920791]
22. Magnuson K, Jackowski S, Rock CO, Cronan JJE. Microbiol. Rev. 1993; 57:522. [PubMed: 8246839]
23. Massengo-Tiasse RP, Cronan JE. Cell Mol. Life Sci. 2009; 66:1507. [PubMed: 19151923]
24. Ward WH, Holdgate GA, Rowsell S, McLean EG, Pauptit RA, Clayton E, Nichols WW, Colls JG, Minshull CA, Jude DA, Mistry A, Timms D, Camble R, Hales NJ, Britton CJ, Taylor IW. Biochemistry. 1999; 38:12514. [PubMed: 10493822]
25. D A, Grant GA, Barton DH, Jacobs WR Jr, Sacchetti JC. Science. 1998; 279:98. [PubMed: 9417034]
26. Grassberger MA, Turnowsky F, Hildebrandt J. J. Med. Chem. 1984; 27:947. [PubMed: 6379179]
27. Samuel BU, Hearn B, Mack D, Wender P, Rothbard J, Kirisits MJ, Mui E, Wernimont S, Roberts CW, Muench SP, Rice DW, Prigge ST, Law AB, McLeod R. Proc. Natl. Acad. Sci. U. S. A. 2003; 100:14281. [PubMed: 14623959]
28. Tipparaju SK, Muench SP, Mui EJ, Ruzhenikov SN, Lu JZ, Hutson SL, Kirisits MJ, Prigge ST, Roberts CW, Henriquez FL, Kozikowski AP, Rice DW, McLeod RL. J. Med. Chem. 2010; 53:6287. [PubMed: 20698542]

29. Evans DA, Katz JL, West TR. *Tetrahedron Lett.* 1998; 39:2937.
30. Guari Y, van Es DS, Reek JNH, Kamer PCJ, van Leeuwen PWNM. *Tetrahedron Lett.* 1999; 40:3789.
31. Maiti D, Buchwald SL. *J. Org. Chem.* 2010; 75:1791. [PubMed: 20141182]
32. Kuo S-C, Wu J-S, Huang L-J, Wu C-H, Huang S-C, Chou T-C. *J. Heterocycl. Chem.* 1989; 26:605.
33. Fomovska A, Huang Q, El Bissati K, Mui EJ, Witola WH, Cheng G, Zhou Y, Sommerville C, Roberts CW, Bettis S, Prigge ST, Afanador GA, Hickman MR, Lee PJ, Leed SE, Auschwitz JM, Pieroni M, Stec J, Muench SP, Rice DW, Kozikowski AP, McLeod R. *Antimicrob. Agents Chemother.* 2012; 56:2666. [PubMed: 22354304]
34. Muench SP, Prigge ST, McLeod R, Rafferty JB, Kirisits MJ, Roberts CW, Mui EJ, Rice DW. *Acta. Crystallogr. Sect. D.* 2007; 63:328. [PubMed: 17327670]
35. Heath RJ, Li J, Roland GE, Rock CO. *J. Biol. Chem.* 2000; 275:4645.
36. Freundlich JS, Anderson JW, Sarantakis D, Shieh HM, Yu M, Valderramos JC, Lucumi E, Kuo M, Jacobs WR Jr, Fidock DA, Schiehser GA, Jacobus DP, Sacchettini JC. *Bioorg. Med. Chem. Lett.* 2005; 15:5247. [PubMed: 16198563]
37. Yu M, Santha Kumar TR, Nkrumah LJ, Coppi A, Retzlaff S, Li CD, Kelly BJ, Moura PA, Lakshmanan V, Freundlich JS, Vilcheze J-C, Siedner S, Tsai JH-C, Falkard B, Sidhu BS, Purcell LA, Gratraud P, Kremer L, Waters AP, Schiehser G, Jacobus DP, Janse CJ, Ager A, Jacobs WR Jr, Sacchettini JC, Heussler V, Sinnis P, Fidock DA. *Cell Host Microbe.* 2008; 4:567–578. [PubMed: 19064257]

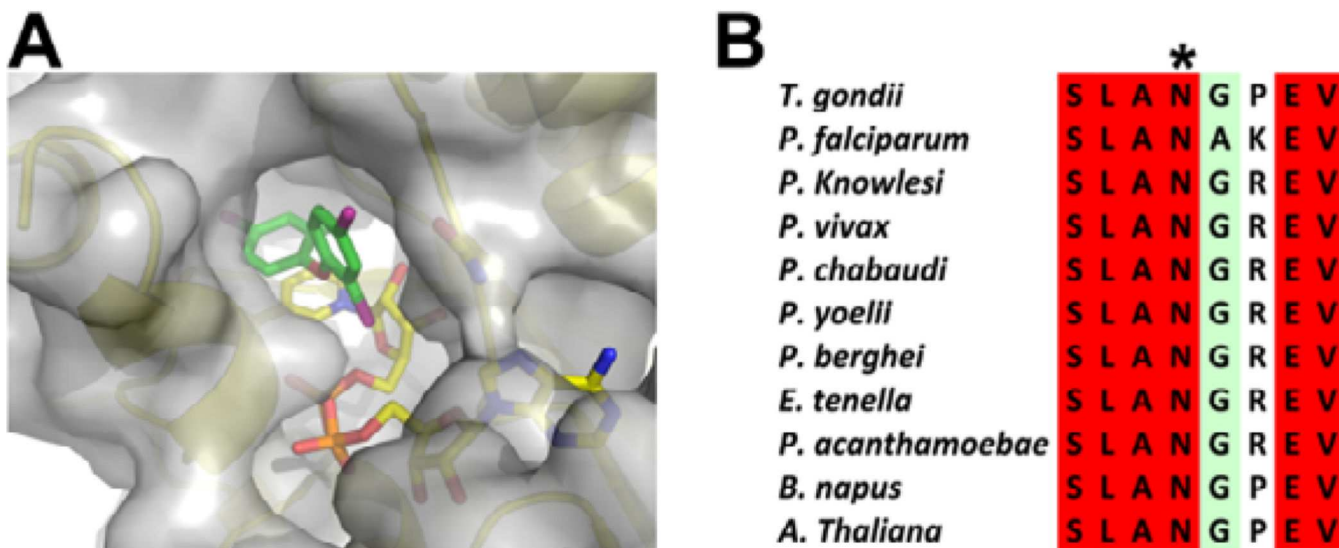


Figure 1.

(A) The TgENR/NAD⁺/triclosan crystal structure shown in a cartoon representation covered by a transparent surface showing the channel which leads from the triclosan inhibitor to the outside solvent. TgENR Asn130 which has been targeted for the design of new inhibitors is shown in stick format close to the B-ring of triclosan. (B) A multiple sequence alignment in the vicinity of TgENR Asn130 (shown by an asterisk) of a number of parasitic and plant ENR enzymes showing full sequence conservation across parasite and plant families.

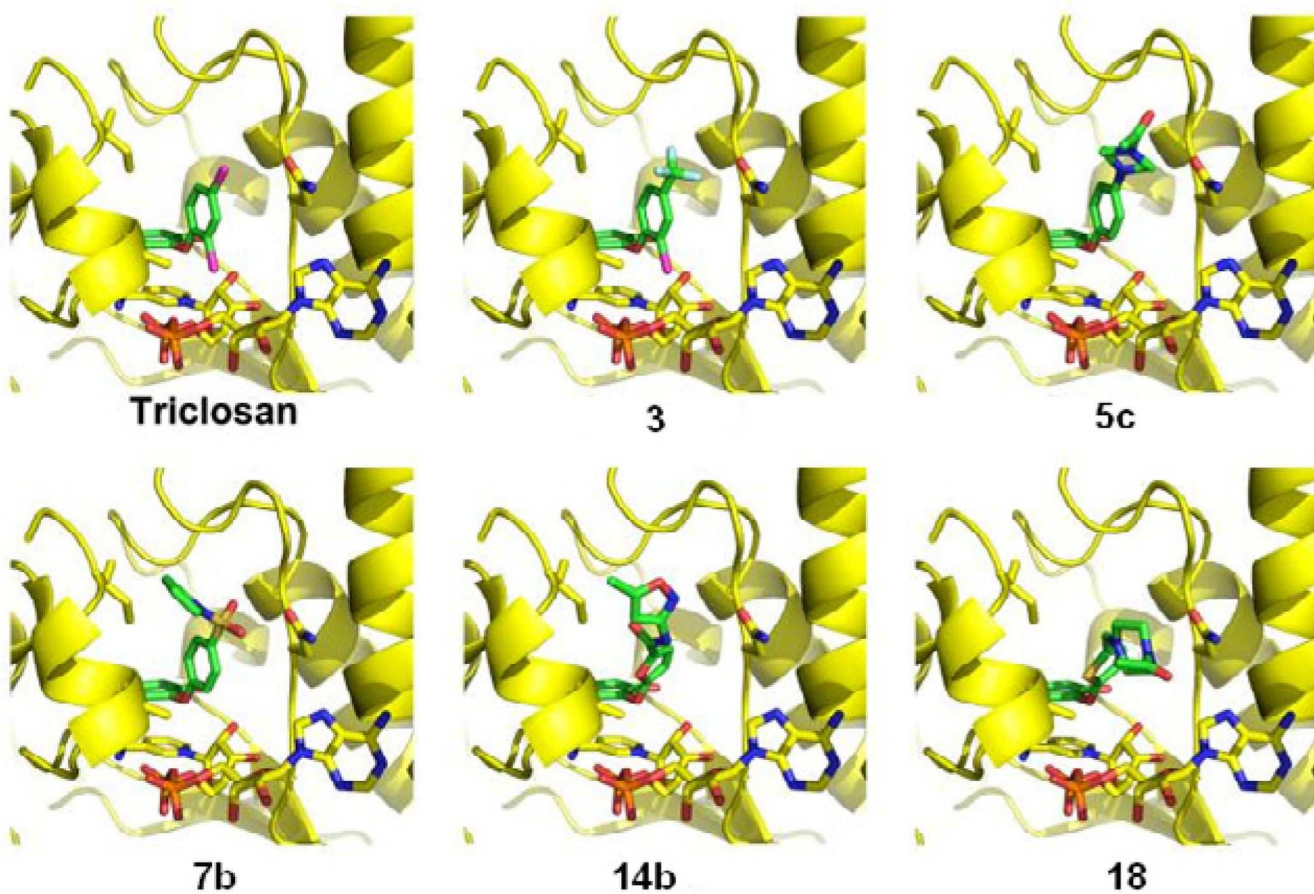


Figure 2. Modeling of the designed inhibitors into the TgENR/NAD⁺/triclosan crystal structure. TgENR is shown in cartoon format with inhibitors, NAD⁺ cofactor, Asn130, Ala231 and Ile235 shown in stick format and colored red, blue, orange, pale blue, magenta, gold, yellow and green for oxygen, nitrogen, phosphorus, fluorine, chlorine, sulfur, carbon (TgENR) and carbon (modeled inhibitors), respectively.

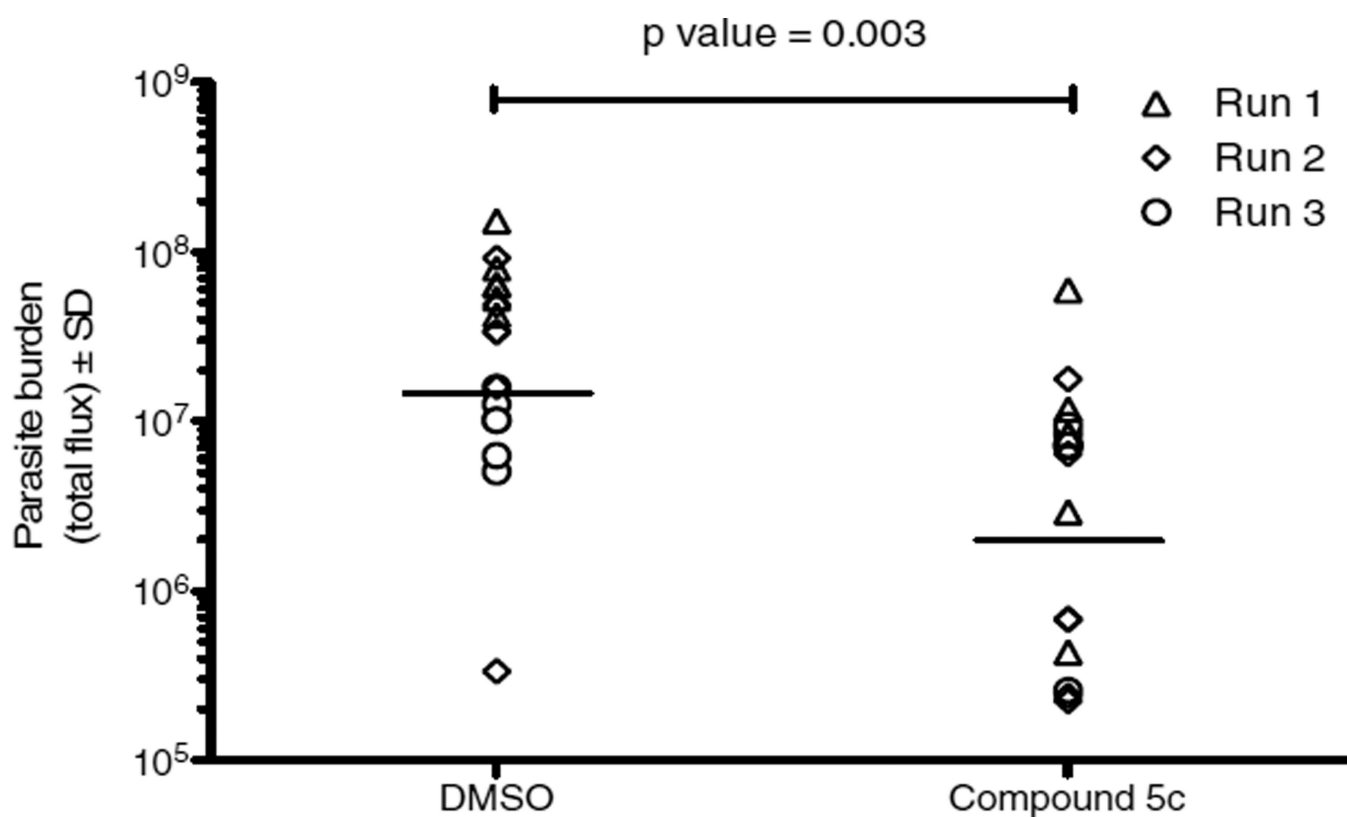
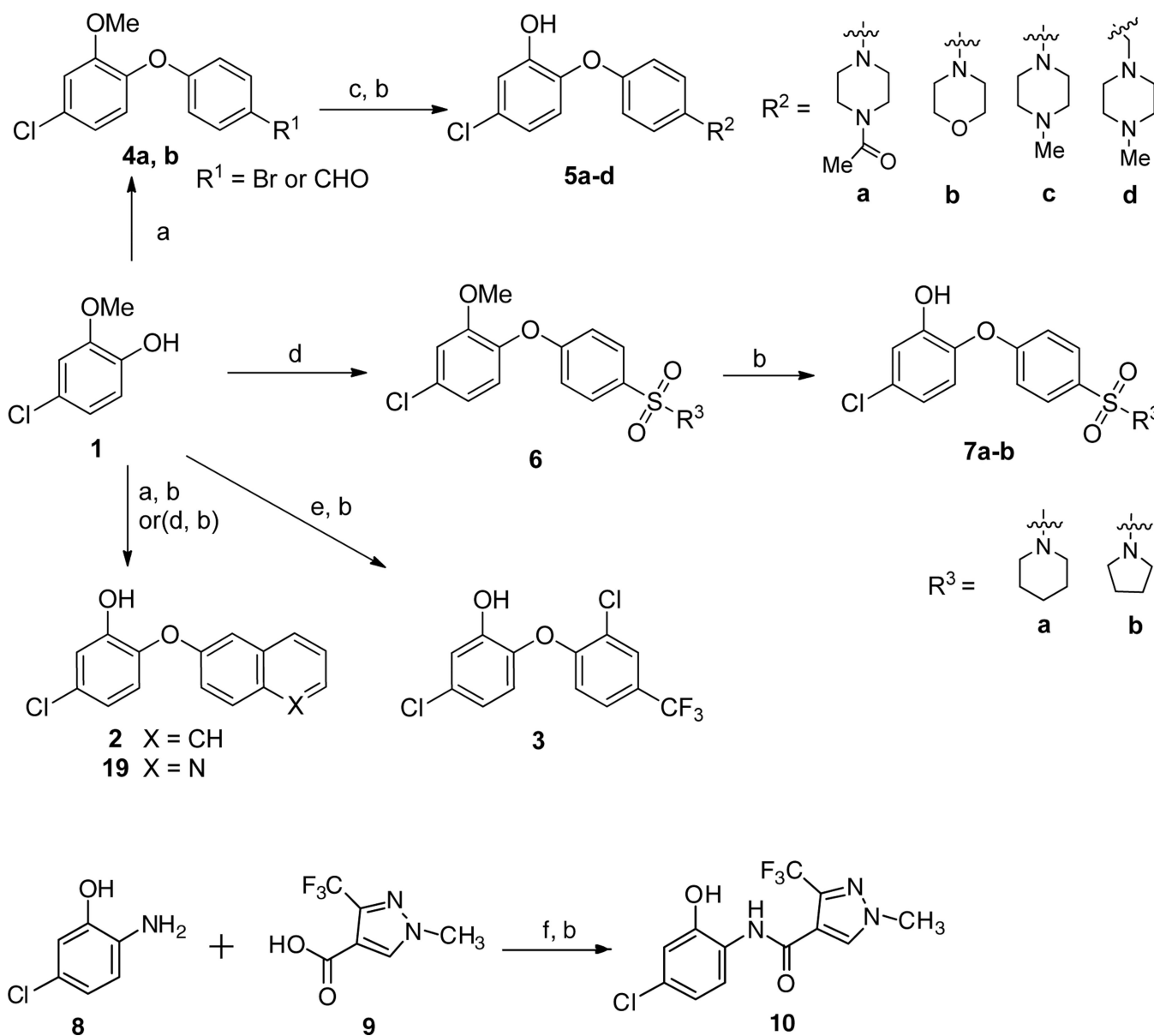
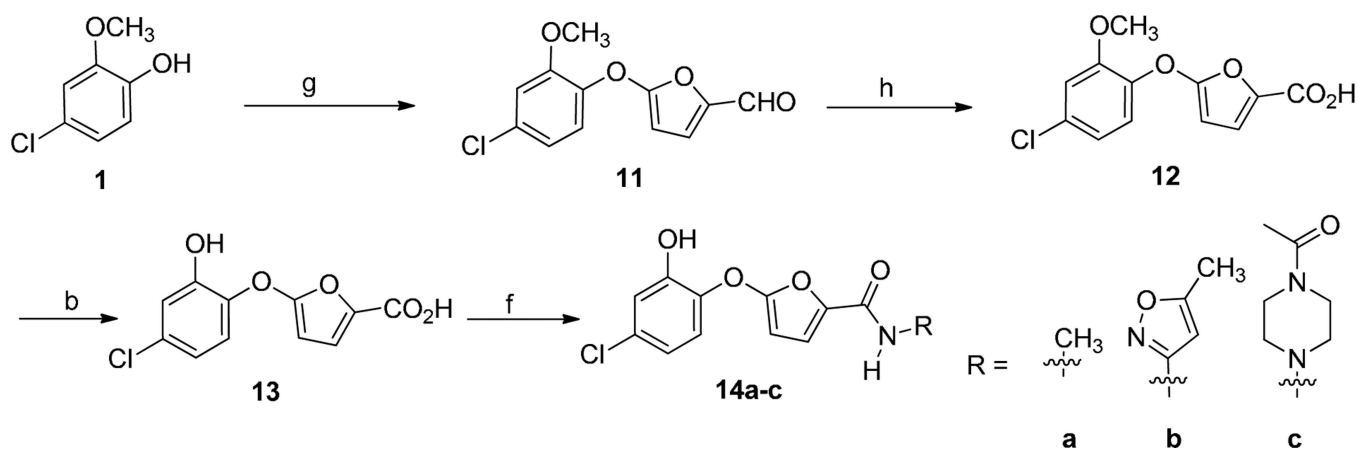


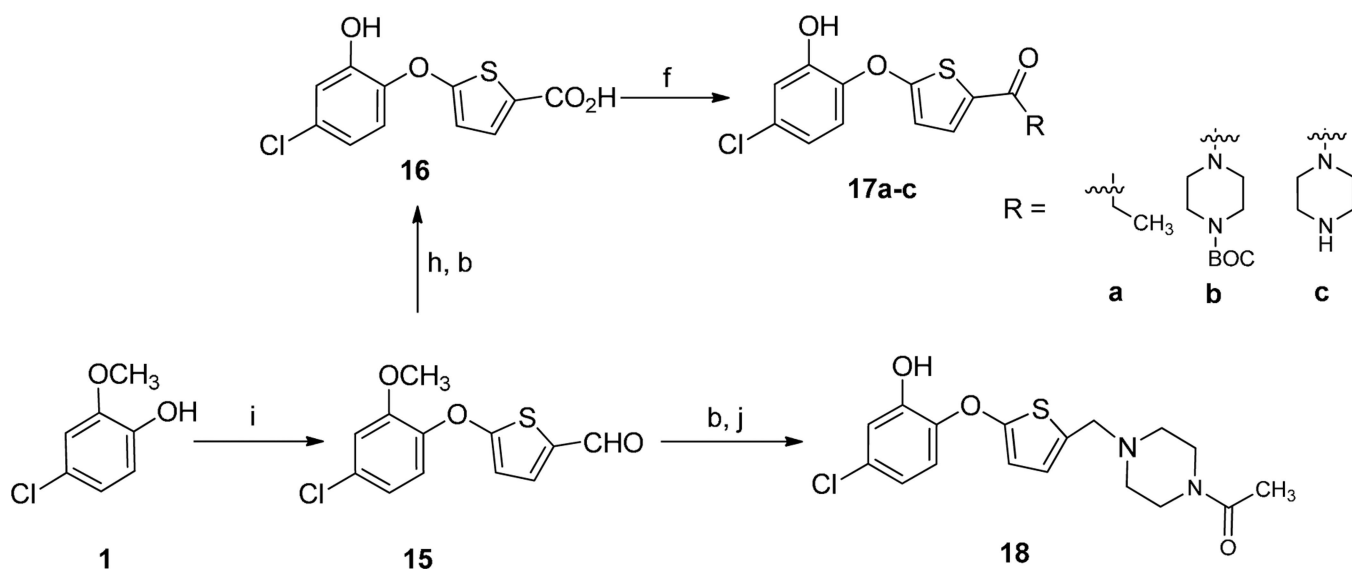
Figure 3. Compound 5c protected mice from *T. gondii* in vivo. Mice were infected intraperitoneally with 2×10^4 tachyzoites (Pru strain expressing firefly luciferase) and imaged daily following intraperitoneal injection of luciferin (3mg) in an IVIS spectrum (Caliper Biosciences).

**Scheme 1.**

Synthesis of 4'-substituted derivatives and compound **10**. Reagents and conditions: (a) arylboronic acid, Et₃N, Cu(OAc)₂, O₂, CH₂Cl₂, room temp, 24 h. For **4a**, R = Br, 70%; for **4b**, R = CHO, 58%; (b) BBr₃, CH₂Cl₂, -78 °C to room temp, 6 h 30%–80%; (c) For R = Br: amine, 3 mol% Pd₂(dba)₃, 9 mol% xantphos, *t*-BuOK, toluene, 110 °C, 5 h; for R = CHO: amine, NaBH(OAc)₃, AcOH, CH₂Cl₂, 0 °C to room temp, 1 h; (d) 4-iodobenzenesulfonamide, 30 mol% picolinic acid, 10 mol% CuI, DMSO, K₃PO₄, 85 °C, 24 h, 60%–70%; (e) 2-chloro-1-fluoro-4-(trifluoromethyl)benzene, K₂CO₃, DMF, 120 °C, 10 h. (f) HOBt, EDCl, DIEA, CH₂Cl₂, 0 °C to rt, overnight.

**Scheme 2.**

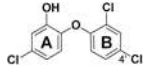
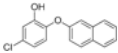
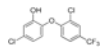
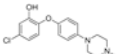
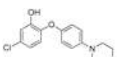
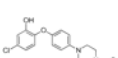
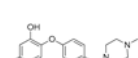
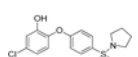
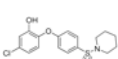
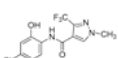
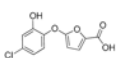
Synthesis of furanyl phenyl ethers. Reagents and conditions: (b) BBr_3 , CH_2Cl_2 , -78°C to room temp, 6 h. 30–80%; (f) amine, HOBt, EDCI, DIEA, CH_2Cl_2 , 0°C to rt, overnight, 40–60%. (g) 5-nitrofur-2-carbaldehyde, NaH, DMSO, 35%; (h) NaClO_2 , DMSO, NaH_2PO_4 , THF/*tert*-BuOH/ H_2O .

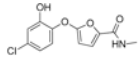
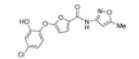
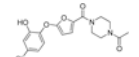
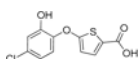
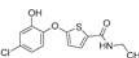
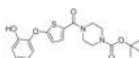
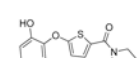
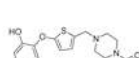
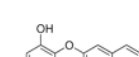
**Scheme 3.**

Synthesis of thienyl phenyl ethers. Reagents and conditions: (b) BBr_3 , CH_2Cl_2 , $-78\text{ }^\circ\text{C}$ to room temp, 6 h; (f) amine, HOBT, EDCI, DIEA, CH_2Cl_2 , $0\text{ }^\circ\text{C}$ to rt, overnight, 40–60%; (h) NaClO_2 , DMSO, NaH_2PO_4 , THF/*t*-BuOH/ H_2O ; (i) 5-bromothiophene-2-carbaldehyde, K_2CO_3 , DMSO, $80\text{ }^\circ\text{C}$, overnight, 60%; (j) amine, $\text{NaBH}(\text{OAc})_3$, AcOH, CH_2Cl_2 , $0\text{ }^\circ\text{C}$ to room temperature, 1 h.

Table 1

Activity data and ADMET parameter predictions for new diaryl ethers inhibitors of TgENR

Compound	Structure	Parasite tissue challenge assay		TgENR Enzyme assay		Calculated ADMET properties (ACD/Labs) ^d					
		MIC ₅₀ (μM)	Toxicity ^a (μM)	Inhibition (%) ^b at 1 μM	IC ₅₀ (nM)	ClogP ^c	TPSA [Å ²]	Sw (mg/L)	Caco-2 (×10 ⁻⁶ cm/s)	F>30%	PPB[%]
Triclosan		5	>10	98	15	5.53	53.25	4.6 ^e	174	0.81	99
2		10	>10	91	41	5.51	29.46	2.8	188	0.81	99
3		4	>10	95	30	5.70	29.46	3.1	192	0.81	99.6
5a		10-1	>10	25	-	3.72	35.94	360	211	0.35	95
5b		>10	>10	74	-	4.15	41.93	78	236	0.35	97
5c		1.5	>10	81	225	2.32	53.01	160	232	0.70	97
5d		>10	>10	32	-	4.64	35.94	1300	188	0.35	89
7a		10	>10	78	-	4.16	75.22	15	227	0.94	97
7b		10	>10	81	-	4.72	75.22	8.3	234	0.94	98
10		>10	>10	27	-	1.54	67.15	270	173	0.95	93
13		>10	>10	79	-	3.25	79.90	710	18	0.91	98

Compound	Structure	Parasite tissue challenge assay		TgENR Enzyme assay		Calculated ADMET properties (ACD/Labs) ^d					
		MIC ₅₀ (μM)	Toxicity ^a (μM)	Inhibition (%) ^b at 1 μM	IC ₅₀ (nM)	ClogP ^c	TPSA [Å ²]	Sw (mg/L)	Caco-2 (×10 ⁻⁶ cm/s)	F>30%	PPB[%]
14a		>10	>10	46	-	2.23	71.70	680	162	0.95	95
14b		10	>10	90	58	3.07	97.73	210	202	0.95	98
14c		>10	>10	45	-	1.72	83.22	1600	140	0.7	95
16		10	>10	48	-	3.84	95.00	610	17	0.91	98
17a		10	10	75	-	3.42	96.80	290	195	0.85	97
17b		>10	>10	76	-	4.71	107.55	130	233	0.35	96
17c		>10	>10	60	-	2.73	90.04	1900	31	0.51	93
18		5	>10	73	965	3.31	81.25	2200	222	0.7	96
19		7.5	>10	87	135	4.22	42.35	21	236	0.81	NA

^aToxicity to human foreskin fibroblasts.

^bAt compound concentration (μM), enzyme inhibition percentage (%).

^cCalculated by ChemDraw Ultra 7.0.

^dThese data were predicted by ADMET suite 5.0 (ACD/Labs). TPSA = topological polar surface area; Sw = solubility in water; Caco-2 permeability; F = probability of bioavailability more than 30%; Log BB = log of the ratio of the drug's concentration in the brain to that in the blood; PPB = plasma protein binding.

^eThe actual water solubility for triclosan is 12 mg/L at 20°C, according to US EPA - Registration Eligibility Decision (RED) for Triclosan.

Table 2Effects on *Plasmodium falciparum* strain D6 (Sierra Leone) and C235 (Thailand; chloroquine resistant)

Compound	SYBR Green D6 IC ₅₀ (ng/ml)	SYBR Green C235 IC ₅₀ (ng/ml)
5c	>10000	>10000