Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 8086–8098
Bioorganic & Medicinal Chemistry

Novel diphenyl ethers: Design, docking studies, synthesis and inhibition of enoyl ACP reductase of *Plasmodium falciparum* and *Escherichia coli*

Manmohan Chhibber,[a] Gyanendra Kumar,[a] Prasanna Parasuraman,[a] T. N. C. Ramya,[a] Namita Surolia[b] and Avadhesha Surolia[a,c,*]

[a]*Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India*
[b]*Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore 560064, India*
[c]*National Institute of Immunology, New Delhi, 110067, India*

Received 1 June 2006; revised 16 July 2006; accepted 18 July 2006
Available online 8 August 2006

Abstract—We designed some novel diphenyl ethers and determined their binding energies for Enoyl-Acyl Carrier Protein Reductase (ENR) of *Plasmodium falciparum* using Autodock. *Out of these*, we synthesized the promising compounds and tested them for their inhibitory activity against ENRs of *P. falciparum* as well as *Escherichia coli*. Some of these compounds show nanomolar inhibition of PfENR and low micromolar inhibition of EcENR. They also exhibit low micromolar potency against in vitro cultures of *P. falciparum* and *E. coli*. The study of structure–activity relationship of these compounds paves the way for further improvements in the design of novel diphenyl ethers with improved activity against purified enzyme and the pathogens.

1. Introduction

Over three billion people live under the threat of malaria and it kills over a million each year—mostly children.[1] Of the four species of the causative agent, *Plasmodium*, of human malaria, *Plasmodium falciparum* causes the most severe and fatal disease—cerebral malaria. Emergence of drug-resistant strains of the pathogen has made the situation worse.[2] Hence, harnessing drug targets unique to the malaria parasite such as the type II fatty acid biosynthesis pathway[3] opens new avenues for drug development against malaria. Type II fatty acid synthesis is brought about by FAS-II (Fatty Acid Synthase II) which is structurally different from the FAS-I (Fatty Acid Synthase I) found in the human host as well as other higher eukaryotes and yeast. While FAS-II has discrete enzymes catalyzing individual reactions of the pathway, FAS-I consists of a single polypeptide with various domains catalyzing all the reactions of the biosynthetic pathway.[4] In *P. falciparum*, type II fatty acid synthesis has been localized in the relict plastid called apicoplast which is evolutionarily related to cyanobacteria.[5] The striking difference in the organization of the enzymes catalyzing fatty acid synthesis in *P. falciparum* and *E. coli* from that in the human host makes it a potent drug target not only for treating malaria but also for many bacterial infections. The iterative cycle of fatty acid biosynthesis consists of four steps—decarboxylative condensation, NADPH-dependent reduction, dehydration and NADH-dependent reduction.[6] The NADH-dependent reduction step is carried out by enoyl-acyl carrier protein (ACP) reductase (ENR) which reduces the trans-2 enoyl bond of enoyl-ACP substrates to saturated acyl-ACPs and plays a deterministic role in completing the fatty acid elongation cycles.[7]

ENR has been validated as a potential antimalarial[3,8] and antibacterial[9] drug target by us and others. We have extensively studied *P. falciparum* ENR (PfENR) using biochemical as well as structural tools and also worked out the mechanism of its inhibition by triclosan.[10–14] Triclosan was found to be effective in killing *P. falcipa-*

Abbreviations: ENR, enoyl ACP reductase; PfENR, *Plasmodium falciparum* enoyl ACP reductase; EcENR, *Escherichia coli* enoyl ACP reductase; FAS, fatty acid synthase; vdw volume, van der Waals volume.

Keywords: Diphenyl ether; Enoyl ACP reductase; *Plasmodium falciparum*; Docking.

* Corresponding author. Tel.: +91 11 26717102/+91 80 22933370; fax: +91 11 26717104/+91 80 23600535; e-mail addresses: surolia@nii.res.in; surolia@mbu.iisc.ernet.in

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.07.034

rum in vitro and curing mice of infection with the rodent malaria species *Plasmodium berghei* as well as acute bacterial infection.[3,15] Triclosan has been traditionally used in consumer products such as toothpaste, mouthwashes, deodorants, soaps, lotions, etc. and it was believed earlier to act by non-specific disruption of the bacterial cell wall. Now, with ENR having been identified as its target, triclosan provides us a promising scaffold, around which we can design newer compounds that can be tested for their possible therapeutic value against malaria and bacterial infections.

The ternary complex of triclosan with NAD^+ and ENR from *P. falciparum* and various other organisms has been studied in detail at the structural level by us and others [14,16] and we have explained how variation in the substrate-binding loop of different ENRs results in variable triclosan affinity.[14] *Mycobacterium tuberculosis* InhA (MtENR) has a comparatively longer substrate-binding loop and Sullivan et al. have exploited it by making 4-substituted triclosan analogues. These alkyl diphenyl ethers with varied alkyl chain lengths significantly increase the affinity of these inhibitors over triclosan in MtENR.[17]

Some triclosan analogues have also been tested by Sivaram et al. and found to have biological activity comparable to triclosan against *E. coli* ENR (EcENR).[18] Recently, 2′- and 4′-substituted triclosan derivatives have been synthesized and tested for their biological activity against PfENR and some compounds found to have inhibitory activities comparable to triclosan.[19,20]

We designed a number of 4-substituted-2′,4′-dichloro and 4-substituted-2′,4′-unsubstituted derivatives of triclosan and calculated their binding energies and binding modes with PfENR using the docking program Autodock. Some of these synthesized compounds had inhibitory activity in nanomolar concentration on purified PfENR and EcENR enzymes as well as low micromolar activity on *P. falciparum* and *E. coli* cultures. Here we describe the synthesis of these compounds and discuss the structure–activity relationship (SAR) with respect to PfENR and EcENR.

2. Results and discussion

We have earlier conducted a detailed study on the nature of the interactions between triclosan and ENR from various sources in order to gain an insight into the features required for a more specific and efficacious inhibitor of ENR.[14] As mentioned earlier, it was clear from the study that though the overall structure of ENR is similar across organisms, the substrate-binding loop has striking differences in ENRs from different species, which correlates with the affinity of the ENR for triclosan. The substrate-binding loops of EcENR and PfENR have a maximum number of interactions with NAD^+ and they have the best binding affinities with triclosan with inhibition constants in the picomolar range. Triclosan binds non-covalently in this loop such that the ether linkage of triclosan is oriented like the enoyl group of the fatty acyl substrate. The ring A of triclosan (Table 1) nestles in a hydrophobic pocket lined by the residues, Tyr267, Tyr277, Gly313, Pro314, Ile323, Phe368, Ile369, and Ala372, while ring B of triclosan is surrounded by the nicotinamide ribose and the phosphates of NAD^+, Met281 and the substrate-binding loop residues Ala319, Ala320, and Ile323 and another loop with conserved residues Ala217, Asn218, Ala219, and Val222.[14]

While the interactions between the substrate-binding loop of the protein and NAD^+ dictate, to an extent, the affinity of the protein for triclosan, there are direct interactions, too, between ENR and triclosan.[14] The phenolic OH of triclosan makes hydrogen bonds with NO2* of nicotinamide ribose and with Tyr277 OH in PfENR which are conserved in other pathogenic organisms too,[14] thus making it very critical for the inhibitory activity. Therefore, the most suitable positions for enhancing the biological activity of diphenyl ether class of compounds can be the substitutions at position 4 in ring A and position 2′ and 4′ in ring B. Examination of the PfENR:NAD^+:triclosan structure has earlier revealed that the 4′-chloro group is in van der Waals contact with the hydrophobic sidechains of Val-222 and Met-281.[16] The halogen is directed toward the sidechain of Asn-218 and the backbone carbonyl of Ala-219, and is approximately 4 Å from the solvent accessible surface, therefore the 4′-position could serve to append functionality to alter the physiochemical properties of the inhibitor.[16] The 2′-chloro group, on the other hand, is in close proximity to Ala-217 and atoms of the pyrophosphate moiety of NAD^+. Series of 2-hydroxy-2′-substituted-4,4′-dichloro and 2-hydroxy-4′-substituted-2′,4-dichloro diphenyl ethers have been synthesized earlier and their inhibitory activity against PfENR determined.[19,20] The current unavailability of data about position 4 of ring A of triclosan made this the obvious target of our study.

We therefore designed and, using Autodock 3.05, docked a number of diphenyl ethers with PfENR to see if these analogues have better binding energies compared to the known inhibitor triclosan. Autodock is a program used to calculate the binding modes and the binding energy of small molecules with macromolecules like proteins and DNA to predict their inhibitory activity. All the compounds docked at the substrate/triclosan binding site and some of them gave binding energies better than or comparable to triclosan, and therefore these compounds were chosen for synthesis (**3**, **6**, **6b**, **9**, **11**, and **13**). In addition, we also synthesized a compound (**2**) that is known to inhibit EcENR[23] as well as two analogues (**19**, **20**) of this compound and tested them on both ENRs. The structures of all these diphenyl ethers and their binding energies as calculated by Autodock are given in Table 1. It is apparent from Table 1 that while substitution of 4-chloro group of triclosan (**1**) with –CHO (**3**), –COOH (**13**), $-CH_2Cl$ (**9**), and $-CH_2OH$ (**11**) gave better binding energies for both PfENR and EcENR compared to that of triclosan, similar derivatives of compound **2** (with no Cl at 2′ and 4′), **19**, and **20** gave binding energies lower than triclosan.

The 2′, 4′-disubstituted diphenyl ethers (**3**, **6**, **6b**, **9**, **11**, and **13**) were synthesized as depicted in Scheme 1. The

Table 1. Binding energies (calculated using Autodock) and inhibitory activities of triclosan and its derivatives with recombinant purified PfENR and EcENR

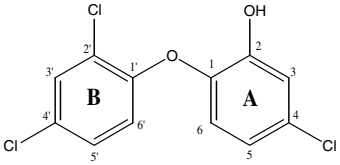
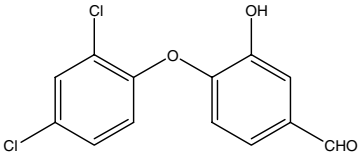
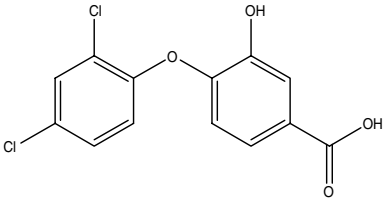
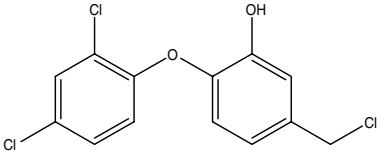
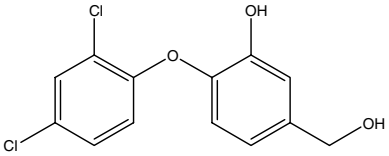
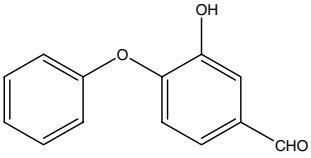
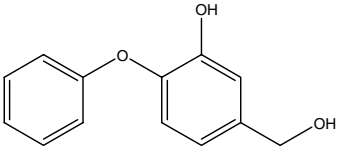
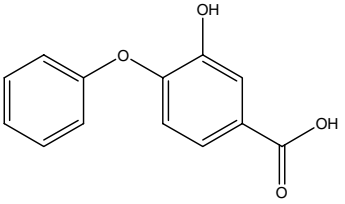
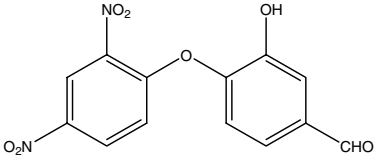
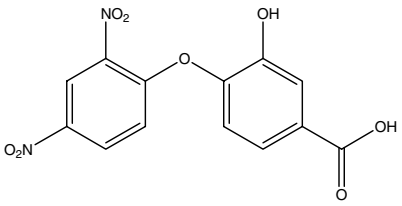
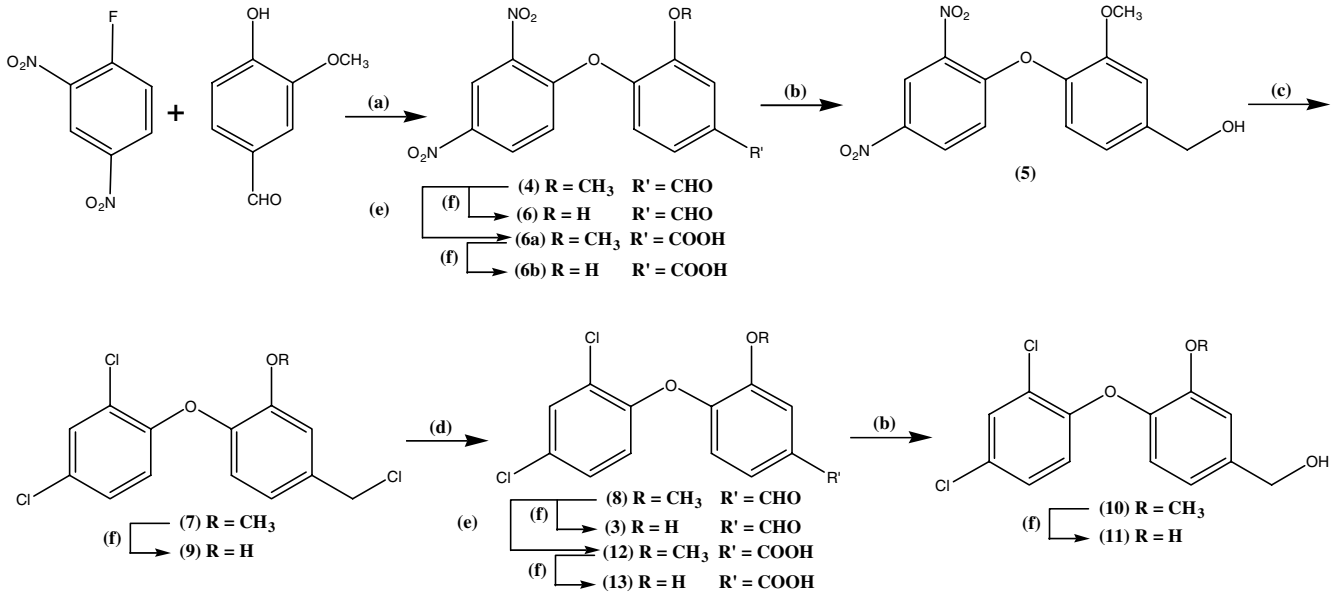
Compound	Structure	(Autodock) PfENR ΔG (kcal/mol)	IC_{50} for PfENR (μM)	K_i for PfENR (μM)	(Autodock) EcENR ΔG (kcal/mol)	IC_{50} for EcENR (μM)	K_i for EcENR (μM)
Triclosan		−11.35	0.20	0.09	−10.26	2.01	0.99
3		−11.55	0.38	0.18	−11.00	1.83	1.00
13		−12.15	0.56	0.21	−11.89	2.25	1.30
9		−11.62	0.70	0.32	−11.00	5.70	2.75
11		−12.00	0.80	0.38	−11.46	3.83	1.85
2		−9.66	0.28	0.13	−9.39	1.30	0.62
19		−10.11	>100	—	−9.87	>100	—
20		−10.28	>100	—	−10.24	>100	—
6		−11.90	74.59	—	−11.03	>100	—

Table 1 (*continued*)

Compound	Structure	(Autodock) PfENR ΔG (kcal/mol)	IC_{50} for PfENR (μM)	K_i for PfENR (μM)	(Autodock) EcENR ΔG (kcal/mol)	IC_{50} for EcENR (μM)	K_i for EcENR (μM)
6b		−12.39	84.57	—	−11.71	>100	—



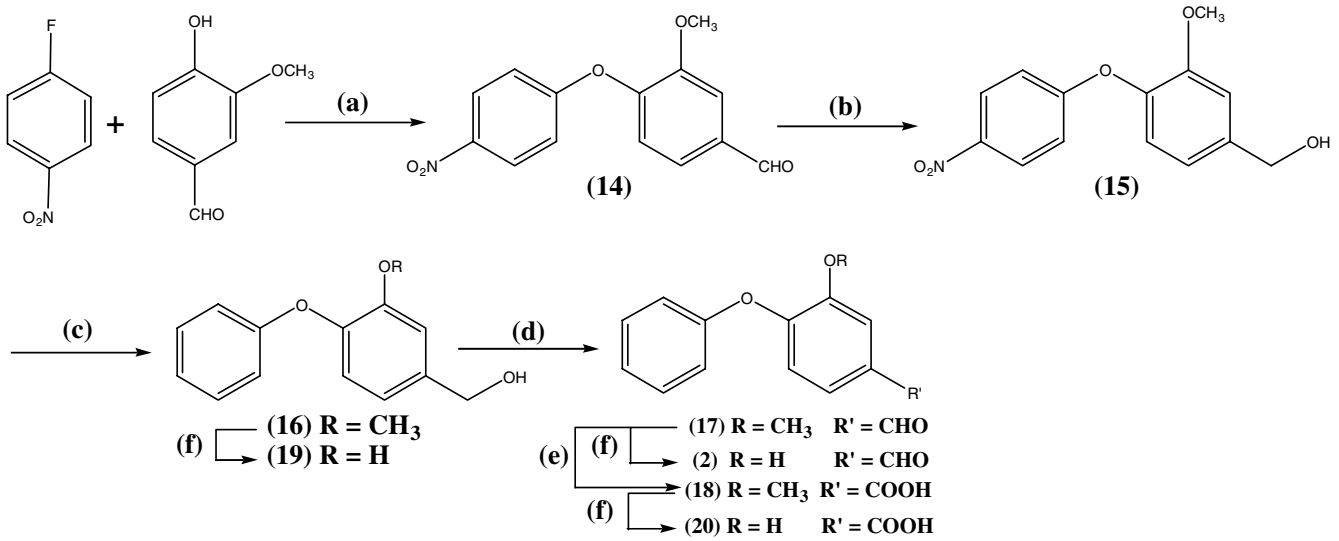
Scheme 1. Reagents: (a) K_2CO_3, DMF, 18-Crown-6; (b) $NaBH_4$, MeOH; (c) i—Fe, $FeSO_4{\cdot}7H_2O$, H_2O; ii—$NaNO_2$, HCl, CuCl–$CuCl_2$; (d) $K_2Cr_2O_7$, H_2O; (e) $KMnO_4$, H_2O; (f) HBr (49% aq), CH_3COOH.

starting diphenyl ether **4** was prepared from commercially available Sanger's reagent (2, 4-dinitrofluorobenzene) and vanillin via nucleophilic aromatic substitution. Benzyl alcohol **5** was synthesized via reduction of aldehyde **4** using $NaBH_4$. The conversion to benzyl alcohol **5** was necessary and used as a protection strategy because reduction of nitro **4** to corresponding amino gave multiple products (as observed in TLC) due to simultaneous presence of nitro and formaldehyde group in **4**. The reduction of nitroarene **5** was done by refluxing in the presence of Fe and $FeSO_4{\cdot}7H_2O$ in water, followed by Sandmayer reaction conditions to get corresponding dichloro derivative **7**. This required presence of both reductant (CuCl) and the ligand transfer agent ($CuCl_2$) in degassed solvent to give dichloro benzyl chloride **7**.[21] Oxidation of **7** with $K_2Cr_2O_7$ in H_2O gave aldehyde **8**. Benzyl alcohol **10** and benzoic acid **12** were prepared by reduction and oxidation of the aldehyde **8** in two separate steps, respectively. Similarly oxidation of aldehyde **4** gave corresponding acid **6a**. Deprotection of the compounds **4**, **6a**, **7**, **8**, **10**, and **12** with 49% aqueous HBr and acetic acid gave corresponding phenolic compounds **6**, **6b**, **9**, **3**, **11**, and **13**, respectively.

The other diphenyl ethers (**2**, **19**, and **20**) were synthesized by a similar protocol (Scheme 2). Nitroarene **15** was prepared by the same reaction sequence as described for compound **5** in Scheme 1. Fe/$FeSO_4{\cdot}7H_2O$-mediated reduction of **15** followed by reductive deamination with ^{t}BuONO in DMF afforded compound **16**.[22] PDC oxidation of benzyl alcohol **16** gave aldehyde **17** which on further oxidation yielded benzoic acid **18**. The intermediates **16**–**18** were treated with HBr (Aq)/Acetic acid to give the final phenols **2**, **19**, and **20**. All compounds were analyzed by ^{1}H and ^{13}C NMR, ESI-Mass, and elemental analysis before assessment of biological activity.

2.1. The effect of substitution at position 4: A study of the 2-hydroxy-4-substituted 2′,4′-dichlorodiphenyl ether series

In the ternary complex, PfENR-NAD^+-Triclosan, ring A stacks over the nicotinamide ring of NAD^+ making π–π interactions.[14] The chlorine at position 4 in this ring is closeted in the pocket created by Tyr267, Phe368, Ile369, Pro314, and Ala372. Our designed derivatives in which this chlorine was substi-



Scheme 2. Reagents: (a) K_2CO_3, DMF, 18-Crown-6; (b) $NaBH_4$, MeOH; (c) i—Fe, $FeSO_4 \cdot 7H_2O$, H_2O; ii—^{t}BuONO, DMF; (d) PDC, CH_2Cl_2; (e) $KMnO_4$, H_2O; (f) HBr (49% aq), CH_3COOH.

tuted with –CHO (**3**), –COOH (**13**), $-CH_2Cl$ (**9**), and $-CH_2OH$ (**11**) groups all yielded better binding energies than triclosan as calculated using Autodock (Table 1). Though these compounds did not give biological activities higher than triclosan, the values of K_i for PfENR inhibition were found to be in the nanomolar range. The best among them is compound **3** (vdw volume, 237.6) with a K_i of 180 nM, and interestingly, as the size of the substituent increases in compounds **13** (vdw volume, 239.9) and **9** (vdw volume, 247.5) the inhibitory activity is reduced too, to 211 nM and 319 nM, respectively, with the exception of **11** (vdw volume, 240.7) which does not have the highest vdw volume but still higher than that of triclosan (vdw volume, 231.2) has inhibitory activity of 378 nM (Table 1). Following this observation, it is not surprising that triclosan itself with the smallest group in position 4 (–Cl) is better than any of these derivatives with a K_i of 99 nM (Table 1). Our results thus far indicate that size of the substituent is one of the factors influencing the affinity of the inhibitors, if not the only factor.

With EcENR, compounds **3**, **13**, **9**, and **11** showed inhibition in the low micromolar range (Table 1). Substitution of –Cl at position 4 of triclosan with –CHO did not significantly change the K_i, the K_i values being 990 nM and 1 μM for triclosan and compound **3**, respectively (Table 1). Substitution with –COOH however increased the K_i to 1.3 μM in compound **13**. The trend of decreasing inhibitory activity with increasing size of the substituent at position 4 was therefore similar to that observed with PfENR. Replacement of –Cl (vdw volume, 231.2) by $-CH_2Cl$ (vdw volume, 247.5) or $-CH_2OH$ (vdw volume, 240.7) increased the value of K_i further to 2.75 and 1.85 μM, respectively (Table 1). Thus, results from all the 2-hydroxy-4-substituted 2′, 4′-dichlorodiphenyl ether series demonstrated the importance of a small group at position 4.

2.2. Effect of –Cl at 2′ and 4′ positions: A study of the 2-hydroxy-4-substituted diphenyl ether series

Having established the role of position 4 of ring A of triclosan, we looked at the effect brought about by the –Cl on positions 2′ and 4′ of triclosan. Series of 4′- and 2′-substituted triclosan derivatives have been synthesized earlier and their inhibitory effect on ENR determined.[19,20] None of the 4′-substituted triclosan derivatives was equivalent or better than triclosan in terms of ENR inhibition.[20] However, X-ray crystal structures of some of these 4′-substituted derivatives of triclosan bound to PfENR in the presence of cofactor demonstrated hydrogen-bonding interactions of the 4′ substituents, nitro, aniline, methylamide and urea, with residues in the active site, Asn 218 and Asn 219.[20] Freundlich et al. therefore speculated that upon replacement of the chloro at 4′ with more polar groups such as aniline, nitro, carboxamido or ureido, the ability to append hydrogen-bonding functionality occurs concomitant with a loss of hydrophobic interactions with Val-222 and Met-281, which are essential for PfENR inhibition.[20] The 2′ position of triclosan, on the other hand, is directed toward the pyrophosphate moiety of NAD^+ and only 4 Å away from two of the negatively charged oxygens.[19] Consequently, triclosan derivatives with hydrophobic amine substituents on the 2′-position demonstrated better inhibitory activity than triclosan.[19]

As part of this study, we looked at the effect of –Cl at 2′ and 4′ positions on 2-hydroxy-4-substituted diphenyl ethers. Biological activity testing demonstrated that compound **2** (–CHO at position 4 and no substitution at positions 2′ and 4′) inhibited PfENR and EcENR with K_i values of 132 nM and 620 nM, respectively (Table 1). Compound **2** has been previously synthesized and found to inhibit EcENR.[23] We now report that it demonstrates potent inhibitory activity against PfENR that is almost equivalent to triclosan. Therefore, the decrease

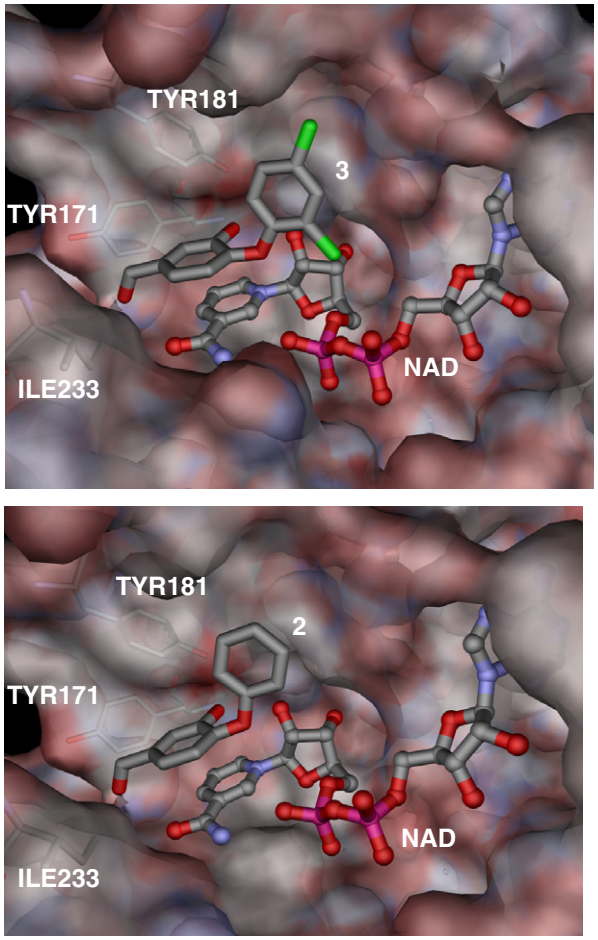


Figure 1. (a) Compound **3** docked with PfENR. (b) Compound **2** docked with PfENR. Inhibitors are shown in sticks. NAD is shown in balls and sticks. Amino acids are shown in thin sticks. Solvent accessible surface is rendered transparent. Atoms colors are: Carbon, grey; Oxygen, red; Nitrogen, blue, Chlroine, green; Hydrogens are not shown for the sake of clarity. Figures were generated in Weblab Viewerlite and rendered with PovRay.

in activity induced by the substitution of –Cl at position 4 by a bigger group, –CHO (in **3**), could be offset by the elimination of the –Cl groups at positions 2′ and 4′ (in **2**) (Fig. 1). Surprisingly, the other two compounds in this series, **19** (with $-CH_2OH$) and **20** (with –COOH), did not inhibit PfENR or EcENR even at 100 μM (Table 1). This suggests that compound **2** was a unique case wherein elimination of –Cl groups at positions 2′ and 4′ led to increased inhibitory activity. The case of compounds **19** and **20** emphasizes the role of –Cl at positions 2′ and 4′. In the absence of these –Cl groups, only compound **2** (–CHO at 4-position) remained active.

2.3. Effect of $-NO_2$ at 2′ and 4′ positions: A study of the 2-hydroxy-4-substituted 2′,4′-dinitrodiphenyl ether series

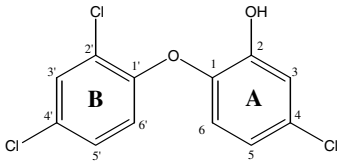
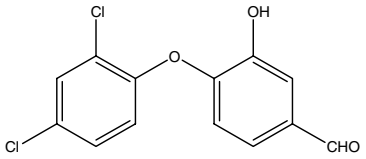
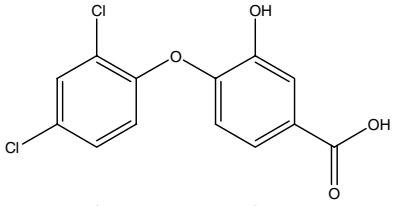
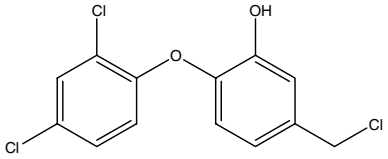
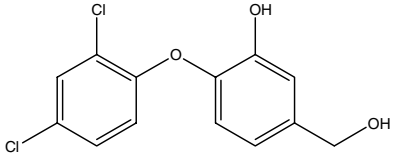
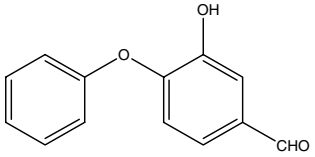
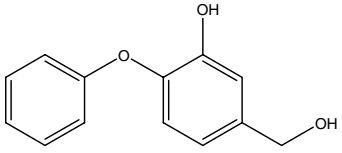
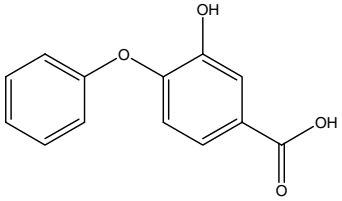
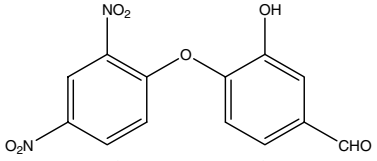
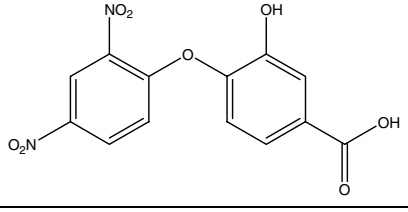
We next looked at the effect of $-NO_2$ substitution at the 2′ and 4′ positions of ring B on the 4-substituted derivatives. Both compounds in this series, **6** (with –CHO substitution on position 4 and $-NO_2$ on positions 2′ and 4′) and **6b** (with –COOH substitution on position 4 and $-NO_2$ on positions 2′ and 4′) showed low micromolar activity. The IC_{50}s of these compounds for PfENR were 74.59 and 84.57 μM, respectively (Table 1). As for EcENR, compounds **6** and **6b** did not inhibit EcENR enzyme activity even up to 100 μM (Table 1). K_i values were hence not determined. Compared to the series of compounds where ring B did not have any 4′ and 2′ substitutions, compound **6** fared poorly as compared to compound **2**, while compound **6b** fared better than compound **20**. Thus, the addition of the two nitro groups at 2′ and 4′ positions in compound **6b** contributed to a slight gain of activity against ENR which was lost in the previous series due to removal of –Cl at these positions. However, both **6** and **6b** had poor inhibitory activity as compared to compounds **3** and **13**, which only differ from these compounds in having –Cl groups instead of $-NO_2$ groups at positions 2′ and 4′, thus emphasizing the significant role played by –Cl at positions 2′ and 4′.

On the whole, out of the nine compounds that were synthesized, seven (**3**, **13**, **9**, **11**, **2**, **6**, and **6b**) showed good to moderate activity (Table 1). Five of these compounds (**3**, **13**, **9**, **11**, **2**) gave IC_{50} values in low micromolar range against EcENR also (Table 1). On cluster analysis of the docked conformations generated by Autdock, it was found that while high-affinity compounds give one prominent cluster of near-native conformations, conformations of lower affinity compounds get divided into more than one cluster. In this study, for PfENR, the analogues with K_i values comparable to that of triclosan (**2**, **3**, **11**, and **13**) had one prominent cluster of docked conformations, similar to the case of triclosan. But compounds with low inhibitory activity (**6**, **6b**, **19**, and **20**) tended to dock in multiple conformations in the active site (data not shown). Thus, true hits can be distinguished from the false positives on the basis of the distribution of docked conformations among clusters. Another important observation about the performance of Autodock is that it over-estimates the contribution of functional groups attached to an inhibitor in the calculation of binding energy. The calculated binding energies of all the compounds with chlorines at 2′ and 4′ positions are higher than of compounds with no chlorines at these positions. Similarly addition of even bigger groups like $-NO_2$ at 2′ and 4′ positions given higher calculated binding energy, though these compounds do not inhibit the two ENRs in nanomolar concentration.

2.4. Biological activity of the synthesized triclosan derivatives

All the synthesized compounds inhibited *E. coli* cultures with IC_{50} values following a trend similar to that of inhibition of the EcENR enzyme activity. Hence, while triclosan inhibited *E. coli* cultures with an IC_{50} of 750 nM, **3**, **13**, **9**, and **11** inhibited the cultures with IC_{50} values of 14.34, 19.16, 18.45, and 40.04 μM, respectively (Table 2). Compound **2** showed the best growth inhibitory activity with an IC_{50} of 2.00 μM as also reported earlier, and compounds **19**, **20**, **6**, and **6b** inhibited cultures only at concentrations greater than 100 μM (Table 2). With *P. falciparum* cultures, however, no specific trend could be seen. While triclosan inhibited cul-

Table 2. Inhibitory activity of triclosan and its derivatives on cultures of *Plasmodium falciparum* and *Escherichia coli*

Compound	Structure	*P. falciparum* (IC_{50} μM)	*E. coli* (IC_{50} μM)
Triclosan		0.80	0.75
3		20.84	14.34
13		160.72	19.16
9		25.49	18.45
11		14.80	40.04
2		77.08	2.00
19		85.24	>100
20		796.27	>100
6		34.71	>100
6b		—	>100

ture growth with an IC_{50} of 0.8 μM, compounds **11**, **3**, **9**, and **6** showed inhibition in the low micromolar range with IC_{50} values of 14.80, 20.84, 25.49, and 34.71 μM, respectively (Table 2). Compounds **13**, **2**, **19**, **20**, and **6b** inhibited cultures of the malaria parasite only at high micromolar concentrations (Table 2). The problem of delivery across the many membranes enveloping the malaria parasite could be a reason for the poor activity of some of these compounds. Alternatively, it is also possible that some of these compounds which show good activity have other targets too, inside the parasite.

3. Conclusion

A number of novel diphenyl ether compounds were designed, docked to calculate binding energies and binding modes. Some of the promising molecules have been synthesized and assayed for inhibition of PfENR and EcENR as well as cultured *P. falciparum* and *E. coli*. Though none of these synthesized compounds show better inhibitory activity than triclosan, some of them do show nanomolar inhibitory activity with even inhibition of growth of cultured cells in the low micromolar range. The structure–activity relationship provides a base for further exploration of diphenyl ether compounds for their anti-malarial and anti-microbial activity. Several key points have emerged from our analyses, the most important amongst them being that the –Cl at the position 4 in ring A cannot be replaced with a bulky group as it would lead to destabilization of the stacking interactions of this ring with nicotinamide ring of NAD^+. If replaced with another group like –CHO, alterations in the substituents of positions 2′ and 4′ could result in a regain of activity. The importance of the two chlorines at 2′ and 4′ positions varies with the substitution at position 4. Incorporation of these features in future studies could lead to the design of more efficacious inhibitors of PfENR. This study also underlines that Autodock can distinguish inhibitors from non-inhibitors with a moderate success.

4. Experimental

4.1. Docking of inhibitors with PfENR and EcENR

All the docking simulations were done using AutoDock 3.05 program.[24]

4.2. Preparation of the receptor and ligand molecules

The crystal structures of PfENR (PDB Code: 1NHG) by Perozzo et al.[16] and EcFabI (PDB Code: 1QSG) by Stewart et al.[25] were used for docking studies. The structure co-ordinates were converted into mol2 format with MMFF94 charges assigned using the MOE (Molecular Operating Environment) suite of programs.[26] The molto2pdbqs utility (provided with AutoDock program) was used to prepare the input receptor file containing fragmental volume and solvation parameters. Inhibitors were also built using MOE and energy-minimized with MMFF94 charges. The AutoTors utility (provided with AutoDock program) was used to define torsion angles in the ligands prior to docking.

4.3. Docking simulations

Grid maps for docking simulations were generated with 80 grid points (with 0.375 Å spacing) in x, y, and z direction centered in the active site using the AutoGrid utility of AutoDock program. Lennard-Jones parameters 12-10 and 12-6 (supplied with the program package) were used for modeling H-bonds and van der Waals interactions, respectively. The distance-dependent dielectric permittivity of Mehler and Solmajer [27] was used in the calculations of the electrostatic grid maps. The Lamarckian genetic algorithm (LGA) with the pseudo-Solis and Wets modification (LGA/pSW) method was used with default parameters except for the 'maximum number of energy evaluations' which was increased to 1 million from 250 thousand. Hundred independent runs were conducted for each inhibitor.

4.4. Synthesis of compounds

4.4.1. General. Melting points were determined with Buchi apparatus and are uncorrected. Microanalyses were performed on an automated C, H, N analyzer. Mass analysis was done using Electrospray mass spectrometer; Gas chromatography-coupled mass spectrometer, and high-resolution mass spectrometer. ^1H and ^{13}C NMR spectral analyse were performed on 300, 400, and 75, 100 MHz spectrometer, respectively, with tetramethylsilane as the internal standard (δ ppm). The following abbreviations were used to explain the multiplicities: s, singlet; d, doublet; t, triplet; dd, double doublet; m, multiplet, br, broad. Solvents and reagents were purified according to standard laboratory technique.

4.4.2. 4-(2′,4′-Dinitrophenoxy)-3-methoxybenzaldehyde (4). To a solution of 1-fluoro-2, 4-dinitrobenzene (2.6 ml, 20.9 mmol) in DMF (25 ml) were added K_2CO_3 (11.5 g, 83.9 mmol), 3-methoxy-4-hydroxybenzaldehyde (4.0 g, 26.3 mmol), and 18-crown-6 (50 mg, 0.2 mmol). The mixture was stirred at room temperature for 12 h. After the reaction is complete (TLC monitoring), the reaction mixture was diluted with CH_2Cl_2 (100 ml),washed with water (50 ml), 1 N NaOH (3× 10 ml), water (until neutral to litmus paper), brine, and dried over Na_2SO_4. Evaporation of the organic solvent gave yellow solid **4** in 88.2% (6.0 g, mp 132–133 °C) yield. ^1H NMR (400 MHz, $CDCl_3$): δ 3.8 (s, 3H), 6.9 (d, J = 6.9 Hz, 2H), 7.4 (m, 1H), 7.6 (m, 2H), 8.3 (dd, J = 2.8 Hz, 1H), 8.9 (d, J = 2.4 Hz, 1H), 10.0 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 56.3, 111.9, 117.9, 122.1, 122.6, 125.2, 128.6, 135.8, 139.0, 141.9, 146.6, 151.7, 155.3, 190.4. Anal. Calcd for $C_{14}H_{14}N_2O_7$: C, 52.84; H, 3.17; N, 8.80. Found: C, 52.99; H, 3.39; N, 8.56. GC–MS: m/z 318.0$[M]^+$.

4.4.3. 4-(2′,4′-Dinitrophenoxy)-3-hydroxybenzaldehyde (6). To compound **4** (200 mg, 0.62 mmol) in acetic acid (10 ml) was added 48% HBr (1 ml) and heated under

reflux for 2 h. After cooling to room temperature and confirming completion of the reaction (TLC monitoring), acetic acid was evaporated in vacuo. Water (5 ml) was added to dissolve the contents and the aqueous layer extracted with ethyl acetate (3× 10 ml). The combined organic layers were dried over Na_2SO_4. Evaporation of the organic solvent gave product which was purified using SiO_2 column chromatography and solvent (toluene/ethyl acetate = 75:25) to afford a yellow solid **6** in 50.0% (96 mg, mp 209–210 °C) yield. ^{1}H NMR (400 MHz, $CDCl_3$ & DMSO): δ 7.2–7.3 (m, 2H), 7.9 (m, 2H), 8.4–8.5 (m, 1H), 8.9 (m, 1H), 9.9 (s, 1H), 11.9 (br s, 1H); ^{13}C NMR ($CDCl_3$ & DMSO) : δ 118.7, 119.1, 122.6, 124.3, 130.0, 130.7, 131.4, 140.0, 142.0, 142.7, 155.3, 156.2, 190.5. Anal. Calcd. For $C_{13}H_8N_2O_7$: C, 51.33, H, 2.65; N, 9.21. Found: C, 51.47; H, 2.99; N, 9.65. GC–MS: *m/z* 304.0 $[M]^+$.

4.4.4. 4-(2′,4′-Dinitrophenoxy)-3-methoxybenzoic acid (6a). To compound **4** (200 mg, 0.6 mmol) in H_2O (20 ml) was added $KMnO_4$ (149 mg, 0.9 mmol) in H_2O (15 ml) and the reaction mixture refluxed at 60–70 °C. After 2 h, enough of 10% KOH in water was added to make the reaction mixture alkaline. The hot reaction mixture was filtered through Celite and washed with hot water. The combined filtrate and washings were cooled and acidified with dilute HCl until no further precipitate formed. The aqueous solution was extracted with ethyl acetate (3× 60 ml), dried over Na_2SO_4, and concentrated in vacuo. Crude product obtained was purified using SiO_2 column chromatography and solvent (toluene/methanol = 90:10) to afford a white solid **6a** in 35.0% (73 mg,) yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.9 (s, 3H), 6.9 (d, J = 8.5 Hz, 1H), 7.5 (d, J = 8.7 Hz, 1H), 7.6–7.7(m, 2H), 8.6 (d, J = 2.6 Hz, 1H), 9.1 (m, 1H), 11.2 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 56.7, 114.4, 118.4, 118.7, 119.3, 122.3, 124.5, 126.9, 138.2, 142.4, 151.2, 152.9, 157.3, 170.3. Anal. Calcd for $C_{14}H_{10}N_2O_8$: C, 50.31; H, 3.02; N, 8.38. Found: C, 50.45; H, 3.00; N, 8.26; Electrospray-MS: *m/z* 357.0 $[M+Na]^+$.

4.4.5. 4-(2′,4′-Dinitrophenoxy)-3-hydroxybenzoic acid (6b). Following the procedure detailed for **6**, compound **6b** was isolated and purified using SiO_2 column chromatography and solvent (toluene/methanol = 90:10) to afford a pale yellow solid in 44% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 7.0 (d, J = 8.5 Hz, 1H), 7.6 (d, J = 8.7 Hz, 1H), 7.7–7.8 (m, 2H), 8.7 (d, J = 2.6 Hz, 1H), 9.0 (m, 1H), 11.6 (br s, 1H). ^{13}C NMR (CD_3OD): δ 114.9, 118.4, 118.7, 119.3, 122.6, 124.7, 127.0, 138.4, 142.6, 149.6, 152.9, 157.3, 170.3. Anal. Calcd for $C_{13}H_8N_2O_8$: C, 48.76; H, 2.52; N, 8.75. Found: C, 48.69; H, 2.46; N, 8.63; Electrospray-MS: *m/z* 343.0 $[M+Na]^+$.

4.4.6. 4-(2′,4′-Dinitrophenoxy)-3-methoxybenzylalcohol (5). To an ice-cold suspension of **4** (5.8 g, 18.2 mmol) in methanol (120 ml), a solution of $NaBH_4$ (0.8 g, 21.8 mmol) in methanol (20 ml) was added dropwise over a period of 15 min. The reaction mixture changes color to violet during addition. After the reaction is complete (TLC monitoring), the solvent was evaporated in vacuo and reaction quenched with water. It was extracted with diethyl ether (3× 30 ml) and the combined organic layers were washed with water (2× 25 ml), brine and dried over Na_2SO_4. Evaporation of the organic solvent gave crude product which was purified using SiO_2 column chromatography and solvent (pet ether/ethyl acetate = 50:50) to afford a yellow solid **5** in 62.0% (3.6 g, mp 109–110 °C) yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.8 (s, 3H), 4.7 (s, 2H), 6.9 (d, J = 9.3 Hz, 1H), 7.0–7.3 (m, 3H), 8.3 (m, 1H), 8.9 (m, 1H), 10.9 (br s, 1H); ^{13}C NMR ($CDCl_3$): δ 56.0, 64.7, 111.7, 117.3, 119.7, 122.1, 122.4, 128.7, 138.5, 140.5, 141.0, 146.0, 151.0, 156.5. Anal. Calcd for $C_{14}H_{12}N_2O_7$: C, 52.51; H, 3.78; N, 8.75. Found: C, 52.45; H, 3.39; N, 8.56. GC–MS: *m/z* 320.0$[M]^+$.

4.4.7. 4-(2′,4′-Dichlorophenoxy)-3-methoxybenzylchloride (7). To a suspension of **5** (3.4 g, 10.6 mmol) in refluxing H_2O (100 ml) were added Fe (5.95 g, 106.2 mmol) and $FeSO_4{\cdot}7H_2O$ (2.9 g, 10.4 mmol). The reaction mixture was refluxed for 8 h. After cooling to room temperature and confirming the completion of reaction (TLC monitoring), it was filtered through Celite, washed thoroughly with CH_2Cl_2 (2× 75 ml). The combined organic layers were dried over Na_2SO_4. Evaporation of the organic solvent gave corresponding amine. This was confirmed by spotting the product on alumina plate which upon dipping in ninhydrin solution (2% in ethanol) and further heating turned black.

To a solution of $NaNO_2$ (0.71 g, 10.3 mmol) in degassed concd HCl (20 ml) was added above amine in degassed HOAC (10 ml) at 0 °C. Stirring was continued for 20 min at the same temperature, and the reaction mixture then transferred into CuCl (2.05 g, 20.7 mmol) and $CuCl_2$ (3.52 g, 20.7 mmol) in concentrated HCl (20 ml) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. The reaction was quenched by addition of NH_4OH in saturated NH_4Cl until the blue color persisted. The aqueous solution was extracted with CH_2Cl_2 (3× 60 ml), dried over Na_2SO_4, and concentrated in vacuo. Crude product obtained was purified using SiO_2 column chromatography and solvent (neat toluene) to afford pale yellow oil **7** in 22.4% (490 mg) yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.9 (s, 3H), 4.6 (s, 2H), 6.7 (d, J = 8.4 Hz, 1H), 6.8 (d, J = 8.1 Hz, 1H), 6.9 (m, 1H), 7.0 (d, J = 1.8 Hz, 1H), 7.1 (dd, J = 8.7 Hz, 1H), 7.4 (d, J = 2.7 Hz, 1H) ^{13}C NMR ($CDCl_3$): δ 46.0, 56.0, 113.2, 119.0, 119.9, 121.2, 125.02, 127.7, 128.2, 130.2, 134.6, 144.5, 150.8, 151.8. Anal. Calcd for $C_{14}H_{11}C_{l3}O_3$: C, 52.94; H, 3.49; Cl, 33.49. Found: C, 52.47; H, 3.78; GC–MS: *m/z* 316.0 $[M]^+$.

4.4.8. 4-(2′,4′-Dichlorophenoxy)-3-hydroxybenzylchloride (9). Following the procedure detailed for **6**, compound **9** was isolated and purified using SiO_2 column chromatography and solvent (toluene/ethyl acetate = 95:5) to afford a viscous oily liquid in 67% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.4 (s, 2H), 6.6 (d, J = 8.3 Hz, 1H), 6.8 (dd, J = 8.3 Hz, 1H), 7.0 (d, J = 8.8 Hz, 1H), 7.1 (d, J = 1.8 Hz, 1H), 7.2 (dd, J = 8.7 Hz, 1H), 7.5 (d, J = 2.4 Hz, 1H), 10.6 (br s,

1H) ^{13}C NMR ($CDCl_3$): δ 32.8, 117.3, 121.1, 121.4, 126.5, 128.2, 130.2, 130.7, 134.8, 143.6, 146.8, 150.5. Anal. Calcd for $C_{13}H_9Cl_3O_2$: C, 51.43; H, 2.99. Found: C, 51.81; H, 3.07; Electrospray-MS: *m/z* 301.1 $[M-H]^+$.

4.4.9. 4-(2′,4′-Dichlorophenoxy)-3-methoxybenzaldehyde (8). To compound **7** (450 mg, 1.4 mmol) in H_2O (10 ml) were added $K_2Cr_2O_7$ (500 mg, 1.7 mmol), Na_2CO_3 (30 mg, 0.3 mmol) and 18-crown-6 (7 mg, 0.02 mmol).The reaction mixture was refluxed for 8 h. After cooling to room temperature and confirming the completion of reaction (TLC monitoring), it was filtered through Celite, extracted with ethyl acetate (3× 20 ml), and dried over Na_2SO_4. Evaporation of the organic solvent gave crude product which was purified and SiO_2 column chromatography and solvent (toluene/ethyl acetate = 98:02) to afford yellow oil **8** in 98% (440 mg) yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.9 (s, 3H), 6.8 (d, J = 8.0 Hz, 1H), 6.9 (d, J = 8.8 Hz, 1H), 7.2 (dd, J = 8.8 Hz, 1H), 7.4 (dd, J = 8.0 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.53 (d, J = 1.6 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 56.2, 110.9, 117.2, 121.5, 125.5, 128.2, 129.9, 130.1, 130.6, 132.8, 150.2, 150.6, 150.8, 190.7. Anal. Calcd for $C_{14}H_{10}Cl_2O_3$: C, 56.59; H, 3.39. Found: C, 56.42; H, 3.46; Electrospray-MS: *m/z* 319.0 $[M+Na]^+$.

4.4.10. 4-(2′,4′-Dichlorophenoxy)-3-hydroxybenzaldehyde (3). Following the procedure detailed for **6**, compound **3** was isolated and purified using SiO_2 column chromatography and solvent (toluene/ethyl acetate = 95:5) to afford a white solid (mp 61–62 °C) in 61% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.9 (br s, 1H), 6.7 (d, J = 8.7 Hz, 1H), 7.1 (d, J = 9.0 Hz, 1H), 7.3 (m, 2H), 7.5 (d, J = 2.7 Hz, 1H), 7.6 (d, J = 1.8 Hz, 1H), 9.9 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 115.5, 116.5, 122.7, 123.5, 127.3, 128.5, 130.9, 131.3, 132.8, 146.7, 149.1, 149.2, 190.9. Anal. Calcd for $C_{13}H_8Cl_2O_3$: C, 55.15; H, 2.85. Found: C, 55.26; H, 2.74; HRMS: *m/z* 282.9928 $[M+H]^+$. Found: 282.9937.

4.4.11. 4-(2′,4′-Dichlorophenoxy)-3-methoxybenzylalcohol (10). Following the procedure detailed for **5**, compound **10** was isolated and purified using SiO_2 column chromatography and solvent (toluene/ethyl acetate = 90:10) to afford a viscous oily liquid in 77% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.8 (s, 3H), 4.6(s, 2H), 5.7 (br s, 1H), 6.7 (d, J = 8.7 Hz, 1H), 6.8 (d, J = 8.4 Hz, 1H), 6.9 (dd, J = 8.0 Hz, 1H), 7.0 (d, J = 1.8 Hz, 1H), 7.1 (dd, J = 8.7 Hz, 1H), 7.4 (d, J = 2.1 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 56.0, 64.1, 113.2, 119.0, 119.9, 121.2, 125.0, 127.7, 128.3, 130.2, 134.6, 144.6, 150.8, 151.8. Anal. Calcd for $C_{14}H_{12}Cl_2O_3$: C, 56.21; H, 4.04. Found: C, 56.13; H, 3.96; Electrospray-MS: *m/z* 321.1 $[M+Na]^+$.

4.4.12. 4-(2′,4′-Dichlorophenoxy)-3-hydroxybenzylalcohol (11). Following the procedure detailed for **6**, compound **11** was isolated and purified using SiO_2 column chromatography and solvent (toluene/ethyl acetate = 90:10) to afford a viscous oily liquid in 52% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.5 (s, 2H), 6.7(d, J = 8.7 Hz, 1H), 6.8–6.9 (m, 1H), 7.0 (d, J = 8.0 Hz, 1H), 7.1 (m, 2H), 7.4 (d, J = 2.0 Hz, 1H), 9.8 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 64.4, 115.6, 118.5, 119.2, 119.7, 125.4, 128.0, 128.9, 130.3, 137.8, 142.4, 147.1, 151.2. Anal. Calcd for $C_{13}H_{10}Cl_2O_3$: C, 54.76; H, 3.54. Found: C, 54.63; H, 3.64; Electrospray-MS: *m/z* 307.0 $[M+Na]^+$.

4.4.13. 4-(2′,4′-Dichlorophenoxy)-3-methoxybenzoic acid (12). Following the procedure detailed for **6**, starting with compound **8**, compound **12** was isolated and purified using SiO_2 column chromatography and solvent (toluene/ethyl acetate = 80:20) to afford a white solid **12** in 57.0% (120 mg, mp 146–147 °C) yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.9 (s, 3H), 6.8 (d, J = 8.4 Hz, 1H), 6.9 (d, J = 8.8 Hz, 1H), 7.2 (dd, J = 8.4 Hz, 1H), 7.4 (d, J = 2.8 Hz, 1H), 7.7 (m, 2H), 11.6 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 56.2, 114.2, 117.6, 121.0, 124.0, 125.3, 126.3, 128.1, 129.8, 130.5, 149.9, 150.1, 150.6, 171.4. Anal. Calcd for $C_{14}H_{10}Cl_2O_3$: C, 53.70; H, 3.22. Found: C, 53.67; H, 3.29; Electrospray-MS: *m/z* 335.0 $[M+Na]^+$.

4.4.14. 4-(2′,4′-Dichlorophenoxy)-3-hydroxybenzoicacid (13). Following the procedure detailed for **6**, starting with compound **12**, compound **13** was isolated and purified using SiO_2 column chromatography and solvent (toluene/ethyl acetate = 70:30) to afford a white solid in 64% (mp 152–153 °C) yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.8 (d, J = 8.0 Hz, 2H), 7.1 (m, 1H), 7.5 (m, 2H), 7.7 (s, 1H), 9.1 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 118.2 (Intense), 119.1, 121.1, 124.4, 127.2, 127.6, 128.7, 129.4, 146.0, 147.3, 150.9, 168.5. Anal. Calcd for $C_{13}H_8Cl_2O_4$: C, 52.20; H, 2.70. Found: C, 52.35; H, 2.81; Electrospray-MS: *m/z* 321.0 $[M+Na]^+$.

4.4.15. 3-Methoxy-4-(4′-nitrophenoxy)-benzaldehyde (14). Following the procedure detailed for **4**, compound **14** was isolated in 90% yields. ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.9 (s, 3H), 7.0 (d, J = 6.9 Hz, 2H), 7.2 (d, J = 8.1 Hz, 1H), 7.5 (m, 2H), 8.2 (d, J = 6.9 Hz, 2H), 10.0 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 56.8, 111.7, 115.9, 118.8, 122.7, 124.9, 140.5, 141.8, 143.2, 151.6, 161.4, 189.7. Anal. Calcd for $C_{14}H_{11}NO_5$: C, 61.54; H, 4.06; N, 5.13. Found: C, 61.48; H, 4.16; N, 5.16; Electrospray-MS: *m/z* 296.0 $[M+Na]^+$.

4.4.16. 3-Methoxy-4-(4′-nitrophenoxy) benzyl alcohol (15). Following the procedure detailed for **5**, compound **15** was isolated in 91% (99–100 °C) yield. ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.8 (s, 3H), 4.7 (s, 1H), 6.9 (m, 3H), 7.1 (m, 2H), 8.2 (d, J = 8.1 Hz, 2H), 11.9 (br s, 1H) ^{13}C NMR ($CDCl_3$) δ 55.8, 64.8, 111.7, 115.7, 119.6, 122.4, 125.7, 139.8, 141.9, 142.3, 151.6, 163.5. Anal. Calcd for $C_{14}H_{13}NO_5$: C, 61.09; H, 4.76; N, 5.09. Found: C, 61.12; H, 4.81; N, 5.11; Electrospray-MS: *m/z* 298.0 $[M+Na]^+$.

4.4.17. 3-Methoxy-4-phenoxy benzyl alcohol (16). To a suspension of compound **2** (4.5 g, 16.3 mmol) in refluxing H_2O (100 ml) were added Fe (9.1 g, 162.9 mmol) and $FeSO_4{\cdot}7H_2O$ (9.0 g, 32.4 mmol). The reaction mixture was refluxed for 8 h. After cooling to room temperature, it was filtered through Celite, washed thoroughly with CH_2Cl_2, and dried over Na_2SO_4. The evaporation

of the solvent in vacuo gave corresponding amine. This was confirmed by spotting the product on alumna plate, which upon dipping in ninhydrin solution (2% in ethanol) and further heating turned black.

To a rapidly stirred solution of ^{t}BuONO (1.09 g, 10.6 mmol) in anhydrous DMF (100 ml), heated to 65 °C, was added dropwise via syringe a solution of above amine in DMF (20 ml). The reaction mixture was stirred for 10 min, cooled to room temperature, and diluted with diethyl ether. The resulting solution was poured into 20% aqueous HCl and the organic layer separated. It was washed with dilute HCl (15 ml), H_2O (2× 25 ml), and brine, dried over Na_2SO_4, and evaporated in vacuo to afford a crude mixture which was purified using SiO_2 column chromatography and solvent (EtOAc/toluene = 10:90) to afford a yellow solid **16** in 57% (2.1 g, mp 82–83 °C). ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.8 (s, 3H), 4.6 (s, 2H), 6.8 (d, J = 8.1 Hz, 1H), 6.9 (d, J = 7.5 Hz, 3H), 7.0 (m, 2H), 7.3 (t, J = 8.1 Hz, 2H), 12.1 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 55.8, 64.7, 111.5, 117.0, 119.3, 120.8, 122.4, 129.4, 137.6, 144.2, 151.3, 157.8. Anal. Calcd for $C_{14}H_{14}O_3$: C, 73.03; H, 6.13. Found: C, 72.95; H, 6.23; Electrospray-MS: m/z 253.0 $[M+Na]^+$.

4.4.18. 3-Hydroxy-4-phenoxy benzyl alcohol (19). Following the procedure detailed for **6**, compound **19** was isolated and purified using SiO_2 column chromatography and solvent (toluene/ethyl acetate = 90:10) to afford a white solid (mp 90–91 °C) in 60% yield. ^{1}H NMR (300 MHz; $CDCl_3$) δ 4.6 (s, 2H), 5.9 (br s, 1H), 6.8–6.9 (m, 2H), 7.0–7.1 (m, 4H), 7.3 (t, J = 7.9 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 64.8, 115.0, 117.8, 119.0, 119.1, 123.5, 129.8, 137.6, 142.9, 147.5, 156.8. Anal. Calcd for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59. Found: C, 72.15; H, 5.63; Electrospray-MS: m/z 239.0 $[M+Na]^+$.

4.4.19. 3-Methoxy-4-phenoxy benzaldehyde (17). To a suspension of PDC (294 mg, 0.8 mmol) in CH_2Cl_2 (100 ml) was added **16** (90 mg, 0.4 mmol) in CH_2Cl_2 (20 ml).The reaction mixture was stirred overnight and then filtered through SiO_2 column, washed with ethyl acetate (2× 50 ml). The combined organic layers were dried over Na_2SO_4. Evaporation of the organic solvent gave a viscous liquid **17** in 75% (67 mg) yield. ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.9 (s, 3H), 6.9 (d, J = 8.1 Hz, 1H), 7.0 (d J = 7.8 Hz, 2H), 7.2 (t, J = 7.6 Hz, 1H), 7.4 (m, 3H), 7.5 (s 1H) 9.9 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 56.1, 110.6, 117.7, 119.4, 124.3, 125.7, 129.9, 132.2, 150.9, 152.2, 155.7, 190.9. Anal. Calcd for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.47; H, 5.40; Electrospray-MS: m/z 251.0 $[M+Na]^+$.

4.4.20. 3-Formyl-2-phenoxy phenol (2). Following the procedure detailed for **6**, compound **2** was isolated and purified using SiO_2 column chromatography and solvent (toluene/EtOAc = 95:5) to afford a white solid (mp 76–77 °C) in 61% yield. ^{1}H NMR (300 MHz; $CDCl_3$) δ 6.0 (s, 1H,), 6.8 (d, J = 8.1 Hz, 1H), 7.1 (m, 2 H), 7.2 (m, 1H), 7.3–7.4 (m, 3H), 9.9 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 116.3, 119.7, 123.6, 125.2, 130.2, 132.4, 147.1, 150.0, 154.8, 190.9. Anal. Calcd for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71. Found: C, 72.85; H, 4.76. HRMS: m/z 215.0730 $[M+H]^+$. Found: 215.0704.

4.4.21. 3-Methoxy-4-phenoxy benzoic acid (18). Following the procedure detailed for **6a**, compound **18** was isolated and purified using SiO_2 column chromatography and solvent (toluene/EtOAc = 80:20) to afford a white solid (mp 177–178 °C) in 62% yield. ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.9 (s, 3H), 6.9 (d, J = 8.4 Hz, 1H), 7.0 (d, J = 8.4 Hz, 2H), 7.2 (t, J = 7.4 Hz, 1H), 7.4 (t, J = 7.0 Hz, 2H), 7.6–7.7 (m, 2H), 10.9 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 56.2, 114.0, 118.2, 119.0, 124.0, 124.1, 124.6, 129.9, 150.5, 151.3, 156.3, 171.6. Anal. Calcd for $C_{14}H_{12}O_4$: C, 68.85; H, 4.95. Found: C, 68.76; H, 5.01; Electrospray-MS: m/z 267.0 $[M+Na]^+$.

4.4.22. 3-Hydroxy-4-phenoxy benzoic acid (20). Following the procedure detailed for **6**, compound **20** was isolated and purified using SiO_2 column chromatography and solvent (toluene/EtOAc = 70:30) to afford a white solid (mp 133–134 °C) in 53% yield. ^{1}H NMR (300 MHz; $CDCl_3$) δ 6.9 (d, J = 8.4 Hz, 1H), 7.0 (d, J = 8.0 Hz, 2H), 7.1 (t, J = 7.4 Hz, 1H), 7.3 (t, J = 7.6 Hz, 2H), 7.5 (d, J = 8.0 Hz, 1H), 7.7 (s, 1H), 9.3 (br s, 1H) ^{13}C NMR ($CDCl_3$) δ 117.1, 117.7, 118.3, 120.7, 122.2, 127.0, 128.7, 146.8, 147.5, 156.1, 167.4. Anal. Calcd for $C_{13}H_{10}O_4$: C, 67.82; H, 4.38. Found: C, 67.53; H, 4.51; Electrospray-MS: m/z 253.0 $[M+Na]^+$.

4.4.23. Materials for protein preparation and enzyme assay. Medium components for culture of *Escherichia coli* were obtained from Hi-media (Delhi, India). β-NADH, crotonoyl-CoA, imidazole, and SDS–PAGE reagents were obtained from Sigma. Triclosan was obtained from Kumar Organic Products (Bangalore, India). All other chemicals were of analytical grade. Wild type *E. coli* strain K12 was used. *Plasmodium falciparum* calture medium components RPMI and HEPES, as well as the reagents, orbital, and DMSO were purchased from Sigma. [^{3}H]-hypoxanthine was from Amersham biosciences.

4.4.24. Expression and purification of recombinant PfENR and *E. coli* FabI. PfENR and EcENR were expressed and purified as described previously.[10,7] The plasmids with the 6×-His tagged genes coding for the enzymes were transformed into BL21 (DE3) cells. Cultures were grown at 37 °C for 12 h, followed by subsequent purification on Ni–NTA (Ni^{2+}-nitriloacetate)-agarose column using an imidazole gradient. PfENR and EcENR were eluted using 400 and 200 mM imidazole, respectively. The purity of the protein was confirmed by SDS–PAGE.

4.4.25. Enzyme assay. Enoyl-ACP reductase from both *P. falciparum* and *E. coli* was assayed using spectrophotometric method at 25 °C by monitoring the decrease in the absorbance of NADH at A340. The standard reaction mixture, total volume of 100 μl contained 150 mM Tris–NaCl buffer (pH 7.5), 200 μM crotonoyl-CoA, 100 μM NADH and 1% DMSO. A 10 mM stock solution of the inhibitors was made in DMSO. The stocks were serially diluted to add to the final reaction mixture

for inhibition studies. Data points were the mean of duplicate assays, and the individual values were with in ±10% of the average.

4.4.26. Determination of IC_{50} values. IC_{50} values of the synthesized compounds for ENRs were determined by measuring the ENR activity at various concentrations of these compounds. In the standard reaction mixture mentioned above, various concentrations of the compound of interest were added and the percent inhibition was calculated from the residual enzymatic activity. The percent activity thus calculated was plotted against log concentration of the compound. The data were fitted to a sigmoidal curve by nonlinear regression analysis using Sigma Plot 6.0 and the value of IC_{50} determined by calculating the concentration of the compound which inhibited ENR activity by 50%. Experiments were conducted at least thrice for each compound to confirm reproducibility.

4.4.27. Determination of K_i. For the determination of K_i values of the inhibitors, ENR activity was determined at three fixed concentrations of NADH (50, 100, and 200 μM) while varying the inhibitor concentration from 1 nM to the respective IC_{50} values and keeping crotonoyl-CoA concentration fixed at 200 μM. The data were analyzed using Dixon Plot.[28] Experiments were conducted at least thrice for each compound to confirm reproducibility.

4.4.28. Determination of antimicrobial activity. The whole cell antimicrobial activity of the test compounds was determined by micro dilution method. Ten millimolar stock solutions of triclosan and its derivatives were made in DMSO. Wild type *E. coli* K12 was grown in LB broth until the mid log phase and then diluted to hundred times in the same medium. Hundred microliters of this cell suspension was used to inoculate each tube containing 10 ml of the medium with the inhibitors. The tubes were incubated at 37 °C for 12 h. Turbidity was measured by the OD reading taken at a time gap of every 2 h at 600 nm. The absorbance values were normalized to the solvent-treated control.

4.4.29. Assessment of inhibition of growth of *P. falciparum* and determination of IC_{50} of the compounds by [^{3}H]-hypoxanthine uptake assay. For experiments requiring *Plasmodium* cultures, chloroquine-sensitive *P. falciparum* strain FCK2 (CQ-sensitive; IC_{50} 18 nM) was cultivated in O+ human red blood cells in medium supplemented with O+ human serum by the candle jar method of Trager and Jenson.[29] Cultures were synchronized by 5% sorbitol treatment,[30] and parasites were observed for viability and changes in morphology by standard Giemsa staining.

The semi-automated microdilution technique of Desjardins et al., which is based on [^{3}H]-hypoxanthine uptake by parasites, was used to assess the sensitivity of the parasites to the various inhibitors.[31] Briefly, synchronized parasites were cultured in 96-well plates (Nunc, Copenhagen, Denmark) at 2–3% hematocrit and at an initial parasitemia of 1–2%, with varying concentrations of the inhibitors, and addition of the inhibitor in fresh medium every 24 h for 48 h. All additions were done in duplicate. Inhibitor stocks were made in sterile DMSO and dilutions made such that the final concentration of DMSO in the parasite culture did not exceed 0.1%. Parasites synchronized at the ring stage were cultured in the presence of varying concentrations of the inhibitors for the first 48 h and then incubated with [^{3}H]-hypoxanthine (1 μCi/well) for the next 36 h and harvested. They were then harvested using a Nunc cell harvester onto glass fibers filters, washed and subjected to liquid scintillation counting (Hewlett-Packard). IC_{50}s were calculated from plots of relative percent parasitemia versus log concentration of the respective inhibitors, fitted to non-linear regression analysis using Sigma Plot 2000 software.

Acknowledgments

The authors thank the Department of Biotechnology, Government of India, for the grant to NS and AS. GK and TNCR acknowledge the Council of Scientific and Industrial Research, Government of India, for senior research fellowship. The authors thank Shailendra Kumar Sharma for cloning and expressing the *E. coli* ENR and Shilpi Sharma for assistance in IC_{50} measurements of the compounds in *E. coli* cultures.

References and notes

1. WHO World Malaria Report, 2005, World Health Organization, Geneva, Switzerland <http://rbm.who.int/wmr2005>.
2. Mutabingwa, T. K.; Anthony, D.; Heller, A.; Hallett, R.; Ahmed, J.; Drakeley, C.; Greenwood, B. M.; Whitty, C. J. *Lancet* **2005**, *365*, 1474–1480.
3. Surolia, N.; Surolia, A. *Nat. Med.* **2001**, *7*, 167–173.
4. Smith, S.; Witkowski, A.; Joshi, A. K. *Prog. Lipid Res.* **2003**, *42*, 289–317.
5. Ralph, S. A.; Van Dooren, G. G.; Waller, R. F.; Crawford, M. J.; Fraunholz, M. J.; Foth, B. J.; Tonkin, C. J.; Roos, D. S.; McFadden, G. I. *Nat. Rev. Microbiol.* **2004**, *2*, 203–216.
6. Rock, C. O.; Cronan, J. E. *Biochim. Biophys. Acta* **1996**, *1302*, 1–16.
7. Heath, R. J.; Rock, C. O. *J. Biol. Chem.* **1995**, *270*, 26538–26542.
8. Wiesner, J.; Seeber, F. *Expert Opin. Ther. Targets* **2005**, *9*, 23–44.
9. Zhang, Y. M.; Lu, Y. J.; Rock, C. O. *Lipids* **2004**, *39*, 1055–1060.
10. Kapoor, M.; Dar, M. J.; Surolia, A.; Surolia, N. *Biochem. Biophys. Res. Commun.* **2001**, *289*, 832–837.
11. Kapoor, M.; Mukhi, P. L.; Reddy, C. C.; Krishnasastry, M. V.; Surolia, N.; Surolia, A. *Biochem. J.* **2004**, *381*, 719–724.
12. Kapoor, M.; Mukhi, M. L.; Surolia, N.; Suguna, K.; Surolia, A. *Biochem. J* **2004**, *381*, 725–733.
13. Kapoor, M.; Gopalakrishnapai, J.; Surolia, N.; Surolia, A. *Biochem. J* **2004**, *381*, 735–741.
14. Pidugu, L. S.; Kapoor, M.; Surolia, N.; Surolia, A.; Suguna, K. *J. Mol. Biol.* **2004**, *343*, 147–155.
15. Sharma, S.; Ramya, T. N. C.; Surolia, A.; Surolia, N. *Antimicrob. Agents Chemother* **2003**, *47*, 3859–3866.

16. Perozzo, R.; Kuo, M.; Sidhu, A. S.; Valiyaveettil, J. T.; Bittman, R.; Jacobs, W. R., Jr.; Fidock, D. A.; Sacchettini, J. C. *J. Biol. Chem.* **2002**, *277*, 13106–13114.
17. Sullivan, T. J.; Truglio, J. J.; Boyne, M. E.; Novichenok, P.; Zhang, S.; Stratton, C. F.; Li, H.-J.; Kaur, T.; Amin, A.; Johnson, F.; Slayden, R. A.; Kisker, C.; Tonge, P. J. *ACS Chem. Biol.* **2006**, *1*, 43–53.
18. Sivaraman, S.; Sullivan, T. J.; Johnson, F.; Novichenok, P.; Cui, G.; Simmerling, C.; Tonge, P. J. *J. Med. Chem.* **2004**, *47*, 509–518.
19. Freundlich, J. S.; Yu, M.; Lucumi, E.; Kuo, M.; Tsai, H. C.; Valderramos, J. C.; Karagyozov, L.; Jacobs, W. R., Jr.; Schiehser, G. A.; Fidock, D. A.; Jacobus, D. P.; Sacchettini, J. C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2163–2169.
20. Freundlich, J. S.; Anderson, J. W.; Sarantakis, D.; Shieh, H. M.; Yu, M.; Valderramos, J. C.; Lucumi, E.; Kuo, M.; Jacobs, W. R., Jr.; Fidock, D. A.; Schiehser, G. A.; Jacobus, D. P.; Sacchettini, J. C. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5247–5252.
21. Galli, C. *J. Chem. Soc., Perkin Trans.* **1981**, *2*, 1459–1461.
22. Doyle, M. P.; Dellaria, J. F.; Siegfried, B.; Bishop, S. W. *J. Org. Chem.* **1977**, *42*, 3494–3498.
23. Heath, R. J.; Yu, Y.-T.; Shapiro, M. A.; Olson, E.; Rock, C. O. *J. Biol. Chem.* **1998**, *276*, 30316–30320.
24. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639–1662.
25. Stewart, M. J.; Parikh, S.; Xiao, G.; Tonge, P. J.; Kisker, C. *J. Mol. Biol.* **1999**, *290*, 859–865.
26. Molecular Operating Environment (MOE 2001.07), Chemical Computing Group Inc.,1255 University St., Suite 1600, Montreal, Quebec, Canada, H3B 3X3.
27. Mehler, E. L.; Solmajer, T. *Protein Eng.* **1991**, *4*, 903–910.
28. Dixon, M. *Biochem. J.* **1972**, *129*, 197–202.
29. Trager, W.; Jenson, J. B. *Science* **1976**, *193*, 673–675.
30. Lambros, C.; Vanderberg, J. *J. Parasitol.* **1979**, *65*, 418–420.
31. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.