Bioorganic & Medicinal Chemistry Letters 23 (2013) 3551–3555

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Development of a triclosan scaffold which allows for adaptations on both the A- and B-ring for transport peptides ☆

Stephen P. Muench [a,*], Jozef Stec [b,†], Ying Zhou [c], Gustavo A. Afanador [d], Martin J. McPhillie [e], Mark R. Hickman [f], Patty J. Lee [f], Susan E. Leed [f], Jennifer M. Auschwitz [f], Sean T. Prigge [d], David W. Rice [e], Rima McLeod [c]

[a] School of Biomedical Sciences, University of Leeds, Leeds, UK
[b] Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612, United States
[c] Department of Ophthalmology and Visual Sciences, Pediatrics (Infectious Diseases), Committees on Genetics, Immunology and Molecular Medicine, Institute of Genomics and Systems Biology and The College, The University of Chicago, Chicago, IL 60637, United States
[d] Johns Hopkins School of Public Health, Rm. E5132, 615 N. Wolfe St., Baltimore, MD 21205, United States
[e] Department of Molecular Biology and Biotechnology, The University of Sheffield, Sheffield, UK
[f] Department of Discovery, Division of Experimental Therapeutics Walter Reed Army Institute of Research, Rm 2N61, 503 Robert Grant Avenue, Silver Spring, MD 20910, United states

ARTICLE INFO

Article history:
Received 19 February 2013
Revised 9 April 2013
Accepted 13 April 2013
Available online 24 April 2013

Keywords:
Enoyl reductase
Triclosan
Toxoplasma
Plasmodium

ABSTRACT

The enoyl acyl-carrier protein reductase (ENR) enzyme is harbored within the apicoplast of apicomplexan parasites providing a significant challenge for drug delivery, which may be overcome through the addition of transductive peptides, which facilitates crossing the apicoplast membranes. The binding site of triclosan, a potent ENR inhibitor, is occluded from the solvent making the attachment of these linkers challenging. Herein, we have produced 3 new triclosan analogs with bulky A- and B-ring motifs, which protrude into the solvent allowing for the future attachment of molecular transporters for delivery.

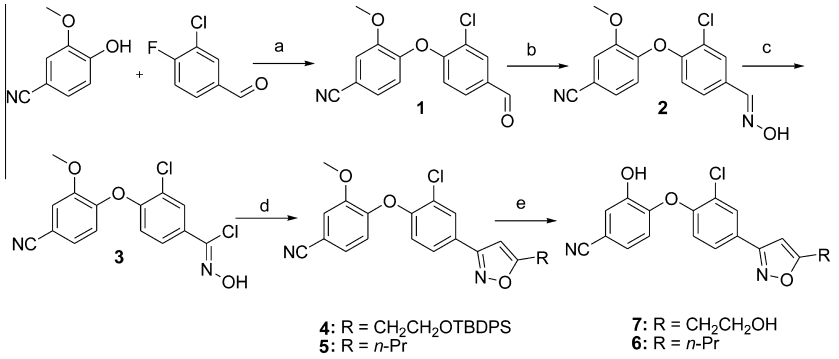
The *Toxoplasma gondii* (*T. gondii*) parasite and other apicomplexans rely on the fatty acid synthesis type II pathway (FAS II), which is prokaryotic-like and distinct from the eukaryotic fatty acid type I pathway (FAS I). FAS II is carried out by discrete mono-functional enzymes, whereas FAS I is typically carried out by one large polypeptide complex.[1,2] This distinction has made this pathway a promising target for anti-microbial drug design.[3,4] The FAS II pathway is composed of four enzymes in an iterative process of fatty acid elongation, in which the enoyl acyl-carrier protein reductase (ENR) has gained the most attention with a range of drugs developed against it. These include the anti-tuberculosis drug isoniazid, the diazaborine family and triclosan which is a common antimicrobial found in, amongst other things, toothpastes, mouthwashes and chopping boards.[5–8] Triclosan has been shown to be a very potent inhibitor which binds at the core of the ENR enzyme, making π stacking interactions with the reduced NAD^+ cofactor.[9] Its binding mode has been characterized as a two state process, where it primarily interacts with the NAD^+ cofactor followed by an α-helix packing over the triclosan, burying it away from the solvent forming a slow tight binding complex.[10] Triclosan is a relatively simple scaffold which has been extensively modified by a number of groups to improve its ADMET properties.

Significant progress has been made toward the development of both *T. gondii* and *Plasmodium falciparum* medicines through the discovery of a FAS II pathway residing within their apicoplast.[11,12] This was particularly pertinent when it was discovered that the *P. falciparum, Eimeria tenella* and *T. gondii* ENR enzyme could be inhibited by the potent antibacterial triclosan.[13–15] Since this discovery, a number of groups have developed a range of triclosan analogs which have shown potent inhibitory effects often with improved ADMET properties.[16–21] Although studies have reported that FASII is not essential for blood stage survival of *P. falciparum* it does play an important role in liver-stage development. Moreover, triclosan may have an off target effect within the blood stage of its lifecycle.[22,23]

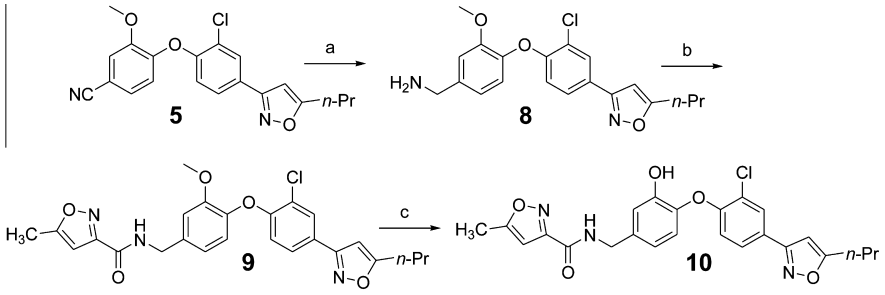
A significant problem with these inhibitors is the need to cross several membranes imposed by the host cell, parasite and

* Corresponding author. Tel.: +44 (0) 113 3434279.
E-mail addresses: s.p.muench@leeds.ac.uk (S.P. Muench), jstec@csu.edu (J. Stec).
† Present address: Chicago State University, College of Pharmacy, DH 203-3, 9501 South King Drive, Chicago, IL 60628, United States. Tel.: +1 773 821 2659; fax: +1 773 821 2595.

0960-894X/$ - see front matter © 2013 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.04.035



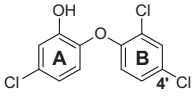
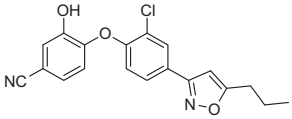
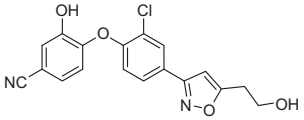
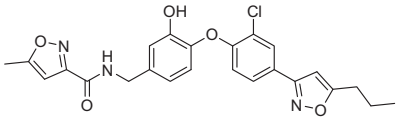
Scheme 1. Synthesis of triclosan analogs bearing isoxazole group on ring B. Reagents and conditions: (a) 3-chloro-4-fluorobenzaldehyde, Cs_2CO_3, DMF, 95 °C, 16 h, 72%; (b) H_2O–EtOH–ice (1:1:2), $H_2NOH{\cdot}HCl$, 50% aq NaOH, rt, 1.5 h, 79%; (c) NCS, DMF, rt, 1.5 h, 100%; (d) sodium ascorbate, $CuSO_4{\cdot}5H_2O$, $KHCO_3$, 1-alkyne, *t*-BuOH–H_2O (1:1), rt, 1 h. For **4**, R = $CH_2CH_2OTBDPS$, 51%; for **5**, R = *n*-Pr, 50%; (e) for **6**: CH_2Cl_2, BBr_3 (4.0 equiv), −78 °C to rt, 3 h, 35%. For **7**: CH_2Cl_2, BBr_3 (8.0 equiv), −78 °C to rt, overnight, 61%.



Scheme 2. Synthesis of triclosan analogs bearing isoxazole groups on ring A and B. Reagents and conditions: (a) (1) $LiAlH_4$, Et_2O, −78 °C to 0 °C, 2.5 h, (2) H_2O, 1.0 M NaOH, 50%; (b) 5-methylisoxazole-3-carboxylic acid, CH_2Cl_2, HOBt, EDCI, Et_3N, rt, 17 h then H_2O, 100% of crude material; (c) CH_2Cl_2, BBr_3 (8.0 equiv), −78 °C to rt within 1 h, then rt for 5 h, 32%.

apicoplast in order to reach the ENR enzyme target. This has been aided with some success through the addition of a cleavable linker and transductive peptide, although further work in this area is needed.[24] In order to establish if a more stable, non releasable molecular transporter can be attached to the A- or B-ring of triclosan in a way that does not significantly alter binding to ENR, we have taken two of our previously successful triclosan modifications which resulted in extensions on the A- and B- ring and combined them. In particular, isoxazole groups were chosen since they retained good potency whilst improving the physiochemical properties (Stec et al., in press). This has resulted in a set of three compounds with potent inhibitory effects and isoxazole extensions,

Table 1
Activity data for new diaryl ethers inhibitors of enoyl reductase

Notebook ID	Structure	Parasite tissue challenge assay		TgENR Enzyme assay		*P. falciparum* blood stage dose response (ng/ml)	
		MIC_{50} (μM)	Toxicity[a] (μM)	Conc. [b](μM) /Inhibition (%)	IC_{50} (nM)	D6	TM91C235
Triclosan		5	>10	98	15	N/A	N/A
6		~4	>10	1/94	29	>2443	>N/A
7		~8	>10	1/94	34	>10,000	>10,000
10		~4	>10	1/89	137	>10,000	>10,000

[a] Toxicity to human foreskin fibroblasts.
[b] At compound concentration (μM), enzyme inhibition percentage (%).

which allow through the incorporation of functional groups to be further utilized for the addition of a linker and transductive peptide or a non-releaseable linker.

The compounds were generated by reacting 4-hydroxy-3-methoxybenzonitrile with 3-chloro-4-fluorobenzaldehyde to give intermediate **1**, which was readily converted to the imidoyl chloride **3** in a two-step protocol.[25] Subsequent reaction of **3** with TBDPS protected 3-butyn-1-ol and 1-pentyne afforded the corresponding isoxazoles **4** and **5**.[26] The final compounds, **6** and **7**, were prepared by the modified demethylation procedure[27] with BBr_3, employing **4** and **5** as the starting materials (Scheme 1).

Reduction of the nitrile **5** provided amine intermediate **8**, which was further elaborated through amide bond formation with 5-methylisoxazole-3-carboxylic acid and demethylation to give the final product **10** (Scheme 2).[28] Full details on compound synthesis are in the Supplementary data.

Inhibitory assays for parasite replication, toxicity against fibroblast host cell tests methods, and enzyme assays were performed as previously described (Stec et al., in press).[21,29–32]

A major hurdle in targeting pathways which reside within the apicoplast is the need for the inhibitor to cross several membrane barriers. In order to avoid this difficulty, we adapted the triclosan scaffold to contain bulky substituents on both the A- and B-rings that were amenable to the addition of non-releasable transport peptides ((Table 1) (Fig. 1A)). The relatively small binding pocket means that these non-releasable linkers must sit outside the cavity, exposed to the solvent to avoid any steric hindrance upon inhibitor binding.

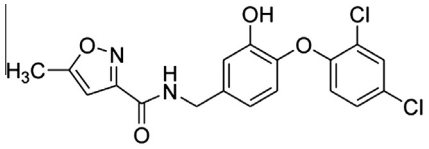


Figure 2. Structural formula of compound **33**.

In the first instance, we used a previously identified modification on the triclosan B-ring whereby a substituted-isoxazole group was added at the 4′-position.[32] This group makes favorable interactions around the entrance of the triclosan binding site and extends out towards the solvent.

In order to test whether further modifications could be placed at the exit of the binding site, the substituent on the isoxazole ring was replaced with either a 5-propyl or 5-ethyl alcohol group. Although only a minimal improvement in the MIC_{50}; from 10 μM to 4 μM (**6**) and 7.5 μM (**7**) is seen, no detrimental effect to the enzymatic activity is observed. Importantly, docking studies have shown that both of these extensions can clearly protrude from the hydrophobic binding site towards a more solvent exposed area of the enzyme (Fig. 1B). Further structural modification of the isoxazole ring could allow for conjugation to a delivery peptide via either a releasable or non-releasable linker.

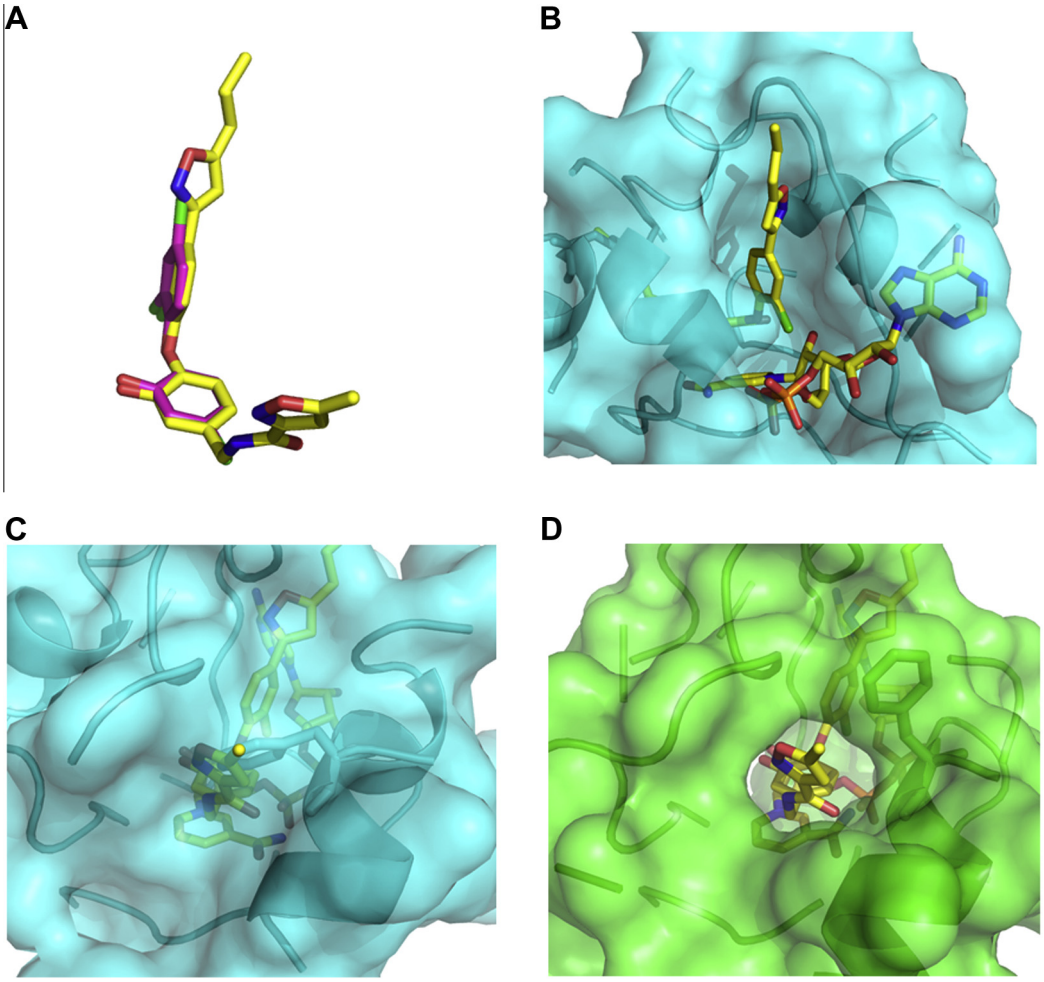


Figure 1. (A) Overlay of triclosan and **10** within the ENR active site showing the similar mode of binding for the common A and B-ring motifs, colored red, blue, green and yellow (**10**) or magenta (triclosan) for oxygen, nitrogen, chlorine and carbon, respectively. (B) Surface view of the modeled TgENR/NAD^+/**10** structure with the modified B-ring protruding into the solvent. (C) Modeling of **10** within the TgENR/NAD^+/triclosan crystal structure where Phe241 adopts a 'closed' position causing steric hindrance. (D) Modeling of **10** within the modified TgENR crystal structure where Phe241 has adopted an 'open' position exposing the A-ring to the solvent.

A more challenging aspect of the project was to produce a modification on the A-ring of triclosan, which occupies an enclosed hydrophobic region, resulting in its exposure to the outside solvent thus allowing for its attachment to a delivery peptide. This is due to the A-ring of triclosan being buried within the binding site, whereas the B-ring is at the base of a channel which leads to the solvent. The tight packing about the A-ring within the ENR enzyme binding site often makes modifications about this ring difficult as there are several residues predicted to make steric clashes with these modified structures, as seen in docking simulations. It is important to note however that most modeling programs do not account for protein flexibility within the binding site.[33]

A solution to this problem was suggested through previous studies of compound **33** (Fig. 2 (Stec et al., in press)). This compound was predicted to bind in a reverse mode to that of triclosan, that is, the A-ring would take the position of the B-ring and vice versa, by the FLEXX docking program. This altered pose was observed due to the large substituent on the A-ring causing severe steric clashes within the binding site which could only be relieved through the reverse binding mode. However, by allowing for flexibility within the active site, in particular the movement of Phe243 about Cβ within the TgENR/NAD^+ complex using the Swiss PDB Viewer the original binding mode was seen.[34] Those orientations that could accommodate the greater steric bulk of our hybrid compounds resulted in a more open binding site such that the A-ring modification is now exposed to the exterior solvent (Fig. 1C and D). We have previously seen the movement of Phe243 about the Cβ, in a manner similar to that of the modeling in a TgENR co-crystal structure for a different family of inhibitors (data not shown). Subsequent docking of the compound series was carried out using AUTODOCK 4.2[35] or MACROMODEL version 8.1[36] and PDB IDs 2O2S[20] and 1LX6[37] available from the RCSB Protein DataBank.

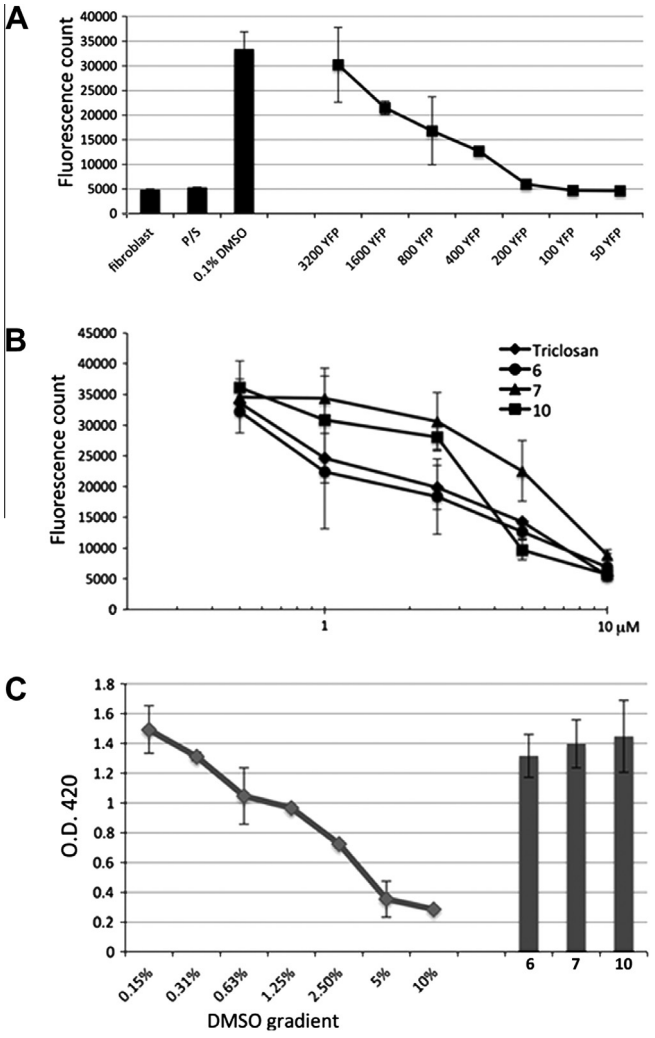


Figure 3. Efficacy and absence of toxicity of compounds against *T. gondii* tachyzoites. (A) Growth of RH-YFP in human Foreskin fibroblasts (HFF), measured as fluorescence intensity. HFF infected with RH-YFP tachyzoites and fluorescence intensities were measured after 72 h. Non infected fibroblasts that provided a baseline control, HFF cells infected with 3200 RH-YFP tachyzoites treated with pyrimethamine/sulfadiazine (p/s) or 0.1% DMSO serve as positive and negative controls respectively. (B) Inhibitory effect of the compounds on RH-YFP. HFF cells were infected with 3200 RH-YFP tachyzoites, compounds at various concentrations were added 1 h after infection. The fluorescence intensities of the samples as reflecting numbers of parasites were measured 72 h after addition of compounds. (C) Effect of the compounds on HFF viability. The viability of host HFF cells was assessed by Wst-1 staining, after 72 h of incubation of compounds at 10 mM concentration. Effect of various concentrations of DMSO present in the HFF culture medium shows varying amounts of toxicity.

A hybrid triclosan scaffold was then designed which contained both A and B ring modifications allowing for the compound to be exposed to the solvent on both ends of the molecular scaffold (**10**). Modeling studies for this compound with increased bulk on both the A- and B-rings does not permit the reverse mode binding seen for the compound **33**, but instead adopts the position shown in Figure 1. Importantly, this compound, despite its bulkier nature, showed no decrease in MIC_{50} value but a slight increase in IC_{50} value from 29 nM (**6**) and 19 nM (compound **33**) to 137 nM (**10**). This increase in IC_{50} to 137 nM is still therapeutically viable and more importantly, the modifications to both the A and B-ring has resulted in a compound which is amenable to further structural modifications to improve both binding and delivery via releasable/non-releasable trans-peptide linkers. In vitro cytotoxicity tests also showed no noticeable increase in toxicity based on the assay used. Growth was measured using a type 1 *T. gondii* parasite tachyzoite RH stably transfected with the yellow fluorescent protein (RH-YFP) gene, with the relative fluorescence intensities of the parasites being directly correlated with parasite viability and numbers (Fig. 3).

The activity of the 3 compounds (**6**, **7** and **10**) were also tested against two different strains of *P. falciparum* (D6 & TM91C235) in a dose-response growth inhibition assay. Only **6** showed modest activity against the drug sensitive strain, D6, but no activity against the drug resistant strain, TM91C235 (Table 1). It is likely that the non-essential nature of the FASII pathway within the blood stage of the *P. falciparum* is responsible for the poor inhibitory effect of these compounds within our assay.[22] Further work will determine the potency of these inhibitors against the liver stage parasite which would be important in stopping recrudescence of the *Plasmodium* parasite.

These results have shown how the triclosan scaffold can be modified to result in both the A- and B-rings being exposed to the exterior solvent without a significant loss in potency or detectable increase in toxicity. This is important since it allows for further structural modifications to be made which are not constrained by the size of the binding site. This also allows for the addition of chemical functionalities which may aid in the delivery of triclosan into the apicoplast, a significant problem in current drug design. Moreover, the bradyzoite form of *T. gondii* is currently impossible to treat with current therapeutics due to the barriers put in place by the cyst form of the parasite. Further work will be carried out to use this scaffold as a basis for modifications by various linker elements which may aid in drug delivery and targeting of a compound whose potency is in the nanomolar range.

Acknowledgments

We thank Dr. Alan Kozikowski for his contributions. The authors are grateful for the financial support provided by NIAID

U01 AI082180-01 and the Rooney-Alden, Taub, Engel, Pritzker, Harris, Kapnick, Mussilami and Samuel families and the Mann-Cornwell Family Foundation. SPM is supported by an MRC Career Development fellowship (G1000567).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.035.

References and notes

1. Smith, S. *FASEB J.* **1994**, *8*, 1248.
2. Magnuson, K.; Jackowski, S.; Rock, C. O.; Cronan, J. E. *Microbiol. Rev.* **1993**, *57*, 522.
3. Heath, R. J.; Rock, C. O. *Curr. Opin. Investig. Drugs* **2004**, *5*, 146.
4. Wright, T. *Curr. Opin. Microbiol.* **2007**, *10*, 447.
5. Baldock, C.; Rafferty, J. B.; Sedelnikova, S. E.; Baker, P. J.; Stuitje, A. R.; Slabas, A. R.; Hawkes, T. R.; Rice, D. W. *Science* **1996**, *274*, 2107.
6. McMurry, L. M.; Oethinger, M.; Levy, S. B. *Nature* **1998**, *394*, 531.
7. Bhargava, N. H.; Leonard, P. A. *Am. J. Infect. Control.* **1996**, *24*, 209.
8. Rozwarski, D. A.; Grant, G. A.; Barton, D. H.; Jacobs, W. R., Jr.; Sacchettini, J. C. *Science* **1998**, *279*, 98.
9. Levy, C.; Roujeinikova, A.; Sedelnikova, S. E.; Baker, P. J.; Stuitje, A. R.; Slabas, A. R.; Rice, D. W.; Rafferty, J. B. *Nature* **1999**, *398*, 383.
10. Ward, W. H.; Holdgate, G. A.; Rowsell, S.; McLean, E. G.; Pauptit, R. A.; Clayton, E.; Nichols, W. W.; Colls, J. G.; Minshull, C. A.; Jude, D. A.; Mistry, A.; Timms, D.; Camble, R.; Hales, N. J.; Britton, C. J.; Taylor, I. W. *Biochemistry* **1999**, *38*, 12514.
11. Goodman, C. D.; McFadden, G. I. *Curr. Drug Targets* **2007**, *8*, 15.
12. McFadden, G. I. *Protoplasma* **2011**, *248*, 641.
13. Lu, J. Z.; Muench, S. P.; Allary, M.; Campbell, S.; Roberts, C. W.; Mui, E.; McLeod, R. L.; Rice, D. W.; Prigge, S. T. *Parasitology* **1949**, *2007*, 134.
14. McLeod, R.; Muench, S. P.; Rafferty, J. B.; Kyle, D. E.; Mui, E. J.; Kirisits, M. J.; Mack, D. G.; Roberts, C. W.; Samuel, B. U.; Lyons, R. E.; Dorris, M.; Milhous, W. K.; Rice, D. W. *Int. J. Parasitol.* **2001**, *31*, 109.
15. Surolia, N.; Surolia, A. *Nat. Med.* **2001**, *7*, 167.
16. Chhibber, M.; Kumar, G.; Parasuraman, P.; Ramya, T. N. C.; Surolia, N.; Surolia, A. *Bioorg. Med. Chem.* **2006**, *14*, 8086.
17. Freundlich, J. S.; Wang, F.; Tsai, H. C.; Kuo, M.; Shieh, H. M.; Anderson, J. W.; Nkrumah, L. J.; Valderramos, J. C.; Yu, M.; Kumar, T. R.; Valderramos, S. G.; Jacobs, W. R., Jr.; Schiehser, G. A.; Jacobus, D. P.; Fidock, D. A.; Sacchettini, J. C. *J. Biol. Chem.* **2007**, *282*, 25436.
18. Maity, K.; Bhargav, S. P.; Sankaran, B.; Surolia, N.; Surely, A.; Suguna, K. *IUBMB Life* **2010**, *62*, 467.
19. Muench, S. P.; Prigge, S. T.; McLeod, R.; Rafferty, J. B.; Kirisits, M. J.; Roberts, C. W.; Mui, E. J.; Rice, D. W. *Acta. Crystallogr., Sect. D* **2007**, *63*, 328.
20. Tipparaju, S. K.; Muench, S. P.; Mui, E. J.; Ruzheinikov, S. N.; Lu, J. Z.; Hutson, S. L.; Kirisits, M. J.; Prigge, S. T.; Roberts, C. W.; Henriquez, F. L.; Kozikowski, A. P.; Rice, D. W.; McLeod, R. L. *J. Med. Chem.* **2010**, *53*, 6287.
21. Perozzo, R.; Kuo, M.; Sidhu, A. b.; Valiyaveettil, J. T.; Bittman, R.; Jacobs, W. R., Jr.; Fidock, D. A.; Sacchettini, J. C. *J. Biol. Chem.* **2002**, *277*, 13106.
22. Yu, M.; Kumar, T. R.; Nkrumah, L. J.; Coppi, A.; Retzlaff, S.; Li, C. D.; Kelly, B. J.; Moura, P. A.; Lakshmanan, V.; Freundlich, J. S.; Valderramos, J. C.; Vilcheze, C.; Siedner, M.; Tsai, J. H.; Falkard, B.; Sidhu, A. B.; Purcell, L. A.; Gratraud, P.; Kremer, L.; Waters, A. P.; Schiehser, G.; Jacobus, D. P.; Janse, C. J.; Ager, A.; Jacobs, W. R., Jr.; Sacchettini, J. C.; Heussler, V.; Sinnis, P.; Fidock, D. A.; Viswanathan, L.; Freundlich, J. S. *Cell Host Microbe* **2008**, *4*, 567.
23. Spalding, M. D.; Prigge, S. T. *Cell Host Microbe* **2008**, *4*, 509.
24. Samuel, B. U.; Hearn, B.; Mack, D.; Wender, P.; Rothbard, J.; Kirisits, M. J.; Mui, E.; Wernimont, S.; Roberts, C. W.; Muench, S. P.; Rice, D. W.; Prigge, S. T.; Law, A. B.; McLeod, R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14281.
25. Xia, Y.; Cao, K.; Zhou, Y.; Alley, M. R.; Rock, F.; Mohan, M.; Meewan, M.; Baker, S. J.; Lux, S.; Ding, C. Z.; Jia, G.; Kully, M.; Plattner, J. J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2533.
26. Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210.
27. Charalambidis, G.; Ladomenou, K.; Boitrel, B.; Coutsolelos, A. G. *Eur. J. Org. Chem.* **2009**, 1263.
28. Liskey, C. W.; Liao, X.; Hartwig, J. F. *J. Am. Chem. Soc.* **2010**, *132*, 11389.
29. Fomovska, A.; Huang, Q.; El Bissati, K.; Mui, E. J.; Witola, W. H.; Cheng, G.; Zhou, Y.; Sommerville, C.; Roberts, C. W.; Bettis, S.; Prigge, S. T.; Afanador, G. A.; Hickman, M. R.; Lee, P. J.; Leed, S. E.; Auschwitz, J. M.; Pieroni, M.; Stec, J.; Muench, S. P.; Rice, D. W.; Kozikowski, A. P.; McLeod, R. *Antimicrob. Agents Chemother.* **2012**, *56*, 2666.
30. Fomovska, A.; Wood, R. D.; Mui, E.; Dubey, J. P.; Ferreira, L. R.; Hickman, M. R.; Lee, P. J.; Leed, S. E.; Auschwitz, J. M.; Welsh, W. J.; Sommerville, C.; Woods, S.; Roberts, C.; McLeod, R. *J. Med. Chem.* **2012**, *55*, 8375.
31. Lai, B. S.; Witola, W. H.; El Bissati, K.; Zhou, Y.; Mui, E.; Fomovska, A.; McLeod, R. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 14182.
32. Cheng, G.; Muench, S. P.; Zhou, Y.; Afanador, G. A.; Mui, E. J.; Fomovska, A.; Lai, B. S.; Prigge, S. T.; Woods, S.; Roberts, C. W.; Hickman, M. R.; Lee, P. J.; Leed, S. E.; Auschwitz, J. M.; Rice, D. W.; McLeod, R. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2035.
33. Cohen, E. M. L.; Machado, K. S.; Cohen, M.; de Souza, O. N. *BMC Genomics* **2011**, *12*, S7.
34. Guex, N.; Peitsch, M. C. *Electrophoresis* **1997**, *18*, 2714.
35. Goodsell, D. S.; Morris, G. M.; Olson, A. J. *J. Mol. Recognit.* **1996**, *9*, 1.
36. MACROMODEL, Version 9.9, Schrodinger, LLC: New York, NY, 2012.
37. Miller, W. H.; Seefeld, M. A.; Newlander, K. A.; Uzinskas, I. N.; Burgess, W. J.; Heerding, D. A.; Yuan, C. C.; Head, M. S.; Payne, D. J.; Rittenhouse, S. F.; Moore, T. D.; Pearson, S. C.; Berry, V.; DeWolf, W. E., Jr.; Keller, P. M.; Polizzi, B. J.; Qiu, X.; Janson, C. A.; Huffman, W. F. *J. Med. Chem.* **2002**, *45*, 3246.