Bioorganic & Medicinal Chemistry Letters 19 (2009) 1982–1985

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Selective inhibition of Pfmrk, a *Plasmodium falciparum* CDK, by antimalarial 1,3-diaryl-2-propenones

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ARTICLE INFO

Article history:
Received 20 December 2008
Revised 9 February 2009
Accepted 10 February 2009
Available online 13 February 2009

Keywords:
Pfmrk
CDK7
Malaria
Plasmodium falciparum
Chalcone
Kinase inhibitors
Cyclin dependent protein kinase
Anti-malarials
Drug target
Parasite

ABSTRACT

The cyclin dependent protein kinases, Pfmrk and PfPK5, most likely play an essential role in cell cycle control and differentiation in *Plasmodium falciparum* and are thus an attractive target for antimalarial drug development. Various 1,3-diaryl-2-propenones (chalcone derivatives) which selectivity inhibit Pfmrk in the low micromolar range (over PfPK5) are identified. Molecular modeling shows a pair of amino acid residues within the Pfmrk active site which appear to confer this selectivity. Predicted interactions between the chalcones and Pfmrk correlate well with observed potency. Pfmrk inhibition and activity against the parasite in vitro correlate weakly. Several mechanisms of action have been suggested for chalcone derivatives and our study suggests that kinase inhibition may be an additional mechanism of antimalarial activity for this class of compounds.

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Cyclin-dependent protein kinases (CDKs) are essential for maintaining the tightly orchestrated cell cycle events associated with growth and development.[1] Abnormalities in the regulation of CDKs lead to numerous cell cycle defects, resulting in disease. To this end, CDKs are pursued as drug targets in cancer, neurological disorders and cardiovascular disease.[2–4] Mammalian CDKs demonstrate variable inhibitor profiles and are often grouped according to their inhibitor sensitivities.[5,6] CDKs are highly conserved among eukaryotic pathogens and their role in cell growth makes them attractive targets for therapeutic intervention. This is especially true for the protozoan parasites responsible for human disease, including *Plasmodium*, *Trypanasoma* and *Leishmania* species.[7–9]

Several CDKs have been characterized from *Plasmodium falciparum*, the causative agent of the most severe form of malaria, including Pfmrk and PfPK5.[10] Pfmrk shares its greatest sequence identity with mammalian CDK7.[11] CDK7 has a dual function as the TFIIH associated kinase responsible for the initiation of transcription and, through T-loop mediated phosphorylation, activation of downstream CDKs.[12] Control of transcription and CDK activation may put CDK7 in a unique position to integrate gene expression with cell cycle control. As with CDK7, maximal Pfmrk kinase activity occurs upon formation of a trimeric complex which includes the effectors proteins PfMAT1 and cyclin. This complex phosphorylates the CTD of RNA Polymerase II.[13] PfPK5 is most similar to mammalian CDK1 and is believed to play a role in regulating DNA synthesis in the malaria parasite.[14] Numerous cyclins associate with and activate PfPK5 in vitro which suggest that cellular activity of PfPK5 may be regulated by its association with a particular cyclin.[15,16] The exact functions of Pfmrk and PfPK5 have yet to be clearly defined; however, substrate specificity, sequence identity and conserved regulatory mechanisms (effector molecule binding and T-loop phosphorylation) suggest that the function of malarial CDKs may also be conserved. If so, they most likely play an essential role in malaria cell cycle control and differentiation and so make an attractive target for antimalarial drug development. Indeed, several different classes of compounds, identified as inhibitors of Pfmrk and PfPK5,[17] are modest inhibitors of *P. falciparum* growth. Herein we explore the chalcones (1,3-diphenyl-2-propenone) a class of compounds that selectivity inhibits Pfmrk over PfPK5.

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0960-894X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2009.02.042

We previously identified the chalcones as Pfmrk inhibitors through an inhibitor-defined pharmacophore specific for Pfmrk.[18,19] Although they do not have a clear mechanism of antimalarial action, initial reports suggest that the chalcones may inhibit malaria proteases responsible for hemoglobin digestion, interfere with the detoxification process of hemozoin formation, inhibit the new permeation pathways that uptake essential nutrients, or a combination of all of these events.[20–22] The identification of chalcones as malaria CDK inhibitors may provide an additional explanation for their antimalarial activity. Herein we expand our chalcone inhibitor studies to define a preliminary SAR for Pfmrk inhibition. Then, by modeling the interaction between these inhibitors and the various CDKs, we explore the amino acid residues in the active site of Pfmrk and identify those which confer the observed selectivity.

A series of diverse chalcone compounds were tested against Pfmrk and PfPK5 (Fig. 1). As shown in Table 1, the extent of inhibition varies among these compounds. The most potent Pfmrk inhibitor was compound **6** with an IC_{50} of 1.3 μM. Several compounds failed to inhibit Pfmrk at concentrations as high as 100 μM (**5**, **15**, **16**, **19**). Failure to effectively inhibit PfPK5 was observed for all tested compounds. The best PfPK5 inhibition was observed at 100 μM with compound **4**. Structural modification, either to introduce complexity or to return to a simpler 'core' chalcone, did not increase PfPK5 inhibition, suggesting that chalcones are not PfPK5 inhibitors.

To better understand the structural features important for the affinity of these compounds for Pfmrk, we have utilized molecular models. We previously developed a 3D model of Pfmrk based on the inactive conformation of hCDK7 (PDB# 1UA2).[23] This model was validated using a series of oxindole-based small molecule inhibitors[24] as described elsewhere.[25] The hydrophilic and π/π stacking interactions predicted between the binding site residues of Pfmrk and the most potent chalcone, **6** (IC_{50} = 1.3 μM), are illus-

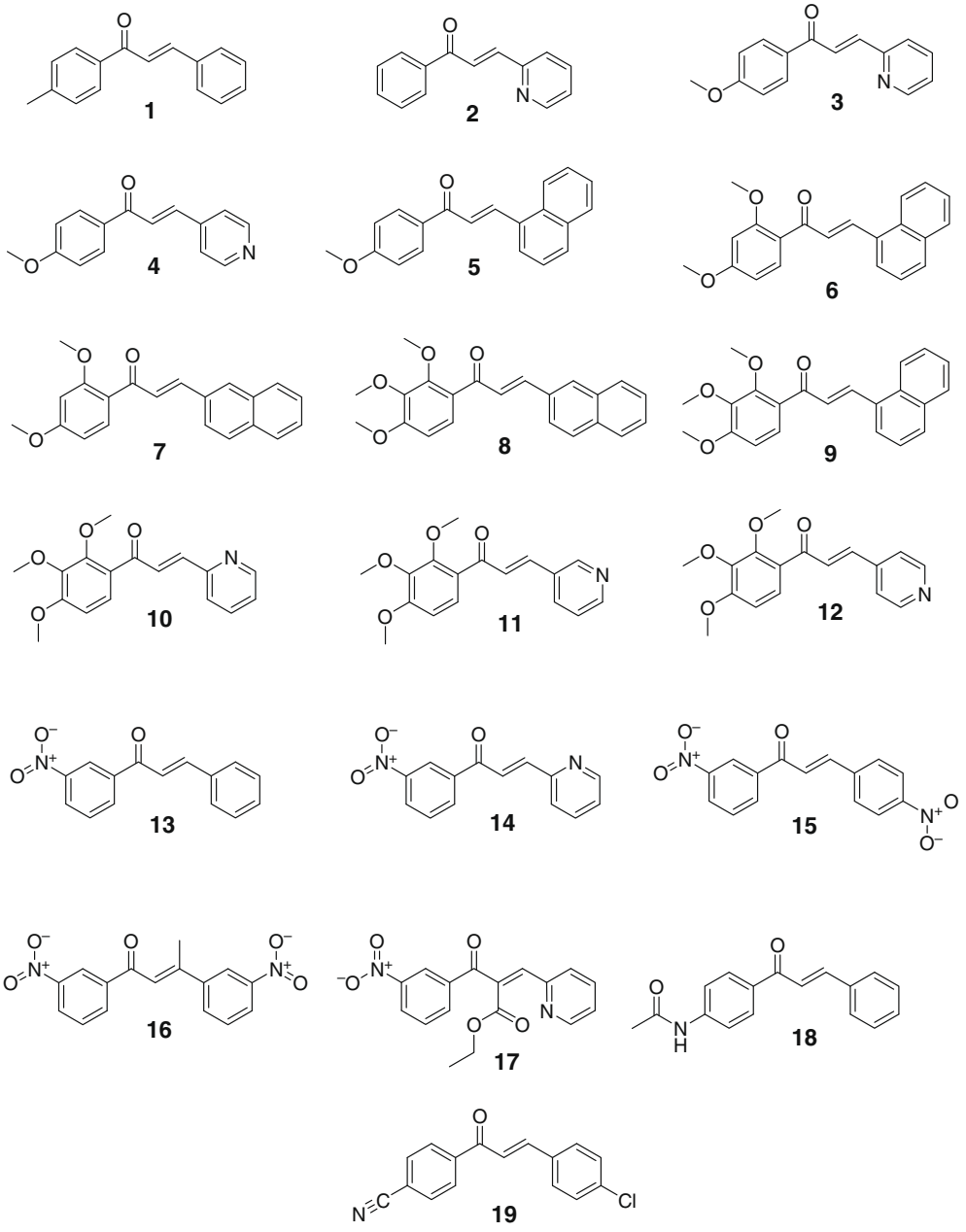

Figure 1. Structures of chalcones examined for Pfmrk inhibition.

Table 1
Inhibitory activity of chalcones on CDKs and parasites in vitro IC_{50} (μM)

Compound	Pfmrk	PfPK5	*P. falciparum* W2	*P. falciparum* D6
1	20	>360	11.5	11.7
2	22	>382	ND	ND
3	12	>100	10.5	7.1
4	8.2	100	12.2	12
5	>100	>100	10	9.1
6	1.3	>100	4.6	4.9
7	100	ND	>16	8.7
8	19	>100	>14	>14
9	50	>100	5.8	4.3
10	34	>100	2.3	2.5
11	38	>100	3.9	3.2
12	9	>100	3.2	2.8
13	10	>316	>19.8	>19.8
14	18	>315	>19.7	11.0
15	268	>268	>16.8	>16.8
16	>256	>256	8.2	8.3
17	61	123	ND	ND
18	17	>302	9.9	10.9
19	>100	>299	10.5	>18.2

ND, not determined.

trated in Figure 2a. In contrast to most kinase inhibitors, the family of chalcone molecules does not mimic ATP by forming hydrogen bonds with hinge region residues (Glu-92 to Met-94). Rather, the active chalcones, through interactions of the ketone carbonyl or para-methoxy groups, form hydrogen bonds with the side chain hydrogen-donating nitrogen of the highly conserved catalytic lysine (Lys-39). Additionally, active compounds form hydrogen bonds with residues that comprise the glycine-rich region of the kinase (Gly-17 to Gly-22). Interestingly, the specific functional group of each small molecule that interacts with the glycine rich region is dependent upon the presence or absence of the pyridine ring which influences the orientation of the inhibitors within the binding site (Fig. 2b). Depending upon the position of the nitrogen within the pyridyl group, the hydrophilic interactions are coordinated by Ser-20, Tyr-21 and Gly-22 (4-pyridyl, e.g., compound **12**) or Gly-19 (3-pyridyl, e.g., compound **11**) and, in some cases, Lys-39 (2-pyridyl, e.g., compound **10**). In the absence of the pyridine ring, methoxy-groups in the *ortho*- and *para*-positions of the phenyl ring are able to substitute as hydrogen bond acceptors for donating residues within the glycine rich region (e.g., compound **6**). The number of polar interactions between Lys-39, the glycine rich region, and a particular chalcone correlates well with the observed affinity of the compound. For example, the dimethoxy-substituted **6** participates in three hydrogen bonds, the trimethoxy-substituted **9** in two, and the monomethoxy **5** in only one. The affinity of these compounds is, respectively, 1.3 μM, 50 μM and >100 μM. The *meta*-methoxy moiety does not participate in a hydrogen bond in any of the structures examined and indeed reduces the likelihood that the *para*-methoxy group is oriented such that a hydrogen bond with Lys-39 is possible. An additional interaction for all active chalcones, regardless of the specific ring system (naphthyl or pyridyl) and of the global orientation of the small molecule within the binding pocket, is a π/π stacking interaction between an aromatic ring of the chalcone and the aromatic side chain of Phe-143. The presence of an aromatic residue at position 143 is unusual (Fig. 3) and is known to effectively bifurcate the ATP binding pocket of Pfmrk, thus decreasing the volume available for association of small molecules.[25,26] Finally, all active compounds participate in a multitude of hydrophobic interactions with nonpolar resides lining the binding site including the residues of the hinge region. Both the α-naphthyl- and β-naphthyl-rings are well tolerated within the hinge region and exhibit similar numbers of hydrophobic interactions. The β-naphthyl connectivity however is not compatible with the methoxy-phenyl mediated hydrogen

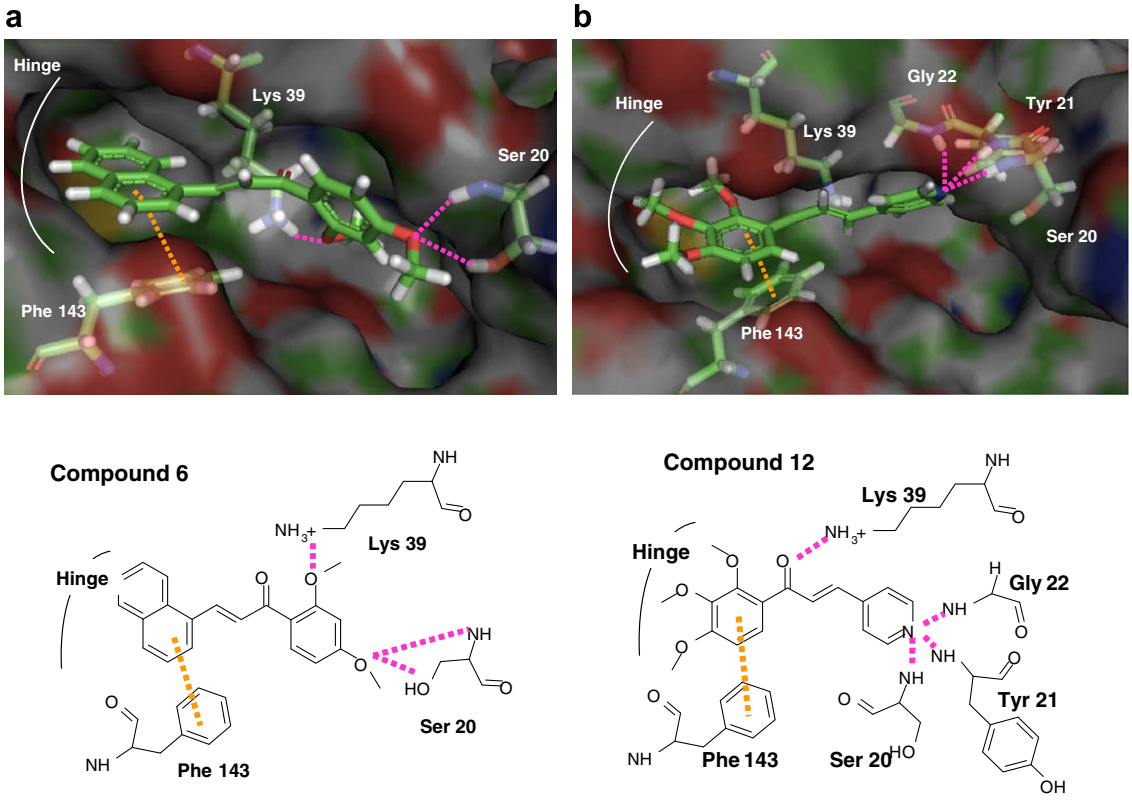

Figure 2. Orientation of chalcones in the Pfmrk binding site dependent upon the presence of a pyridine ring. Upper panel, Pymol representation; Lower panel, flat-land representation. Orange line indicates π/π stacking interactions and magenta line, hydrogen bonds (a) compound **6** and (b) compound **12**.

Figure 3. Multiple sequence alignment of binding site residues. Alignment performed using ClustalW. Numbering for Pfmrk. Boxes indicate the residues that are important for chalcone association with Pfmrk and that are substituted in other kinases. Amino acids in the alignment are not contiguous and only represent those residues that compose the active binding site.

bonds with the glycine rich region, which perhaps explains the 100-fold lower affinity of **7** as compared to **6**. To date, no chalcone tested has appreciable affinity for PfPK5. There are two amino acid residue substitutions within the binding site that we propose contribute to this lack of affinity.

First, as mentioned above, the phenylalanine residue at position 143. In PfPK5, the corresponding residue is leucine (Fig. 3). While leucine does offer potential hydrophobic interactions, the loss of the π/π stacking interactions could result in reduced affinity. Crystal structures for PfPK5 have been solved,[27] and analysis of the chalcone compounds in the PfPK5 kinase binding site provides additional insights into affinity. Compound **6** with the naphthalene ring oriented towards the hinge region is able to potentially form hydrogen bonds with Lys-32 and Lys-88 and participate in numerous interactions with hydrophobic residues (e.g., Phe-79, Val-63 and Leu-132). However, compound **6** is essentially inactive with regard to PfPK5 while it has an appreciable affinity for Pfmrk suggesting the importance of the π/π stacking interaction. Second, Ser-20 within the glycine rich region, is substituted with a threonine in PfPK5. As compounds with higher affinity for Pfmrk generally form hydrogen bonds with Ser-20, the extra methyl group of threonine presumably adversely impacts this association. Interestingly, based on the PfPK5 structural information, Thr-14 is not oriented into the ATP binding site and thus does not provide hydrogen bonding potential.

The activities of these chalcones were evaluated against the chloroquine resistant W2 and sensitive D6 strains of *P. falciparum*. As previously reported,[28] methoxy substitution on the 1-phenyl ring gave the most active antimalarial compounds (Table 1). Generally compounds were equally potent against the W2 and the D6 parasite strains. The only exceptions were compounds **7** and **14** which were less effective against the W2 drug resistant strain. There was a weak correlation between Pfmrk inhibition and antimalarial activity. The most potent Pfmrk inhibitors were the best antimalarial compounds. However several compounds that failed to inhibit Pfmrk, at concentrations as high as 100 μM, were effective at killing the parasites at low micromolar concentrations. In such cases the mechanism of action is clearly not inhibition of Pfmrk. That additional mechanisms of antimalarial activity exist for the chalcones is consistent with previous reports.[20–22]

In summary, we have demonstrated that a series of chalcones preferentially inhibits Pfmrk over PfPK5. Chalcones are known to interfere with cell cycle progression, but to the best of our knowledge, this is the first time that chalcones have been reported as inhibitors of CDKs. They inhibit Pfmrk (selectivity over PfPK5) in the low micromolar range. A weak correlation between Pfmrk inhibition and activity against the parasite in vitro was found. We therefore propose that inhibition of Pfmrk may be an additional mechanism of antimalarial action, at least for some chalcones. Future studies using genetically modified parasite lines that either over-express or knockout Pfmrk may validate this kinase as an in vivo target of chalcones. Such compounds, disclosed in this study, may provide insight not only into the structural differences between PfPK5 and Pfmrk, but also may be used as tools to dissect the role of these cell cycle CDKs during the growth and development of the malaria parasite.

Acknowledgments

The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.042.

References and notes

1. Morgan, D. O. *Annu. Rev. Cell Dev. Biol.* **1997**, *13*, 261.
2. Ivorra, C.; Samyn, H.; Edo, M. D.; Castro, C.; Sanz-Gonzalez, S. M.; Deez-Juan, A.; Andres, V. *Curr. Pharm. Biotechnol.* **2003**, *4*, 21.
3. Hirai, H.; Kawanishi, N.; Iwasawa, Y. *Curr. Top. Med. Chem.* **2005**, *5*, 167.
4. Wang, S.; Fischer, P. M. *Trends Pharmacol. Sci.* **2008**, *29*, 302.
5. Meijer, L. *Prog. Cell Cycle Res.* **1995**, *1*, 351.
6. Fischer, P. M.; Endicott, J.; Meijer, L. *Prog. Cell Cycle Res.* **2003**, *5*, 235.
7. Doerig, C. *Biochim. Biophys. Acta* **2004**, *1697*, 155.
8. Grant, K. M. *Curr. Pharm. Des.* **2008**, *14*, 917.
9. Canduri, F.; Perez, P. C.; Caceres, R. A.; de Azevedo, W. F., Jr. *Curr. Drug Targets* **2007**, *8*, 389.
10. Doerig, C.; Billker, O.; Haystead, T.; Sharma, P.; Tobin, A. B.; Waters, N. C. *Trends Parasitol.* **2008**, *24*, 570.
11. Li, J. L.; Robson, K. J.; Chen, J. L.; Targett, G. A.; Baker, D. A. *Eur. J. Biochem.* **1996**, *241*, 805.
12. Fisher, R. P. *J. Cell Sci.* **2005**, *118*, 5171.
13. Chen, Y.; Jirage, D.; Caridha, D.; Kathcart, A. K.; Cortes, E. A.; Dennull, R. A.; Geyer, J. A.; Prigge, S. T.; Waters, N. C. *Mol. Biochem. Parasitol.* **2006**, *149*, 48.
14. Graeser, R.; Wernli, B.; Franklin, R. M.; Kappes, B. *Mol. Biochem. Parasitol.* **1996**, *82*, 37.
15. LeRoch, K.; Sestier, C.; Dorin, D.; Waters, N.; Kappes, B.; Chakrabarti, D.; Meijer, L.; Doerig, C. *J. Biol. Chem.* **2000**, *275*, 8952.
16. Merckx, A.; Le Roch, K.; Nivez, M. P.; Dorin, D.; Alano, P.; Gutierrez, G. J.; Nebreda, A. R.; Goldring, D.; Whittle, C.; Patterson, S.; Chakrabarti, D.; Doerig, C. *J. Biol. Chem.* **2003**, *278*, 39839.
17. Keenan, S. M.; Geyer, J. A.; Welsh, W. J.; Prigge, S. T.; Waters, N. C. *Comb. Chem. High Throughput Screen* **2005**, *8*, 27.
18. Bhattacharjee, A. K.; Geyer, J. A.; Woodard, C. L.; Kathcart, A. K.; Nichols, D. A.; Prigge, S. T.; Li, Z.; Mott, B. T.; Waters, N. C. *J. Med. Chem.* **2004**, *47*, 5418.
19. Geyer, J. A.; Prigge, S. T.; Waters, N. C. *Biochim. Biophys. Acta* **2005**, *1754*, 160.
20. Dominguez, J. N.; Leon, C.; Rodrigues, J.; Gamboa de Dominguez, N.; Gut, J.; Rosenthal, P. J. *J. Med. Chem.* **2005**, *48*, 3654.
21. Go, M. L.; Liu, M.; Wilairat, P.; Rosenthal, P. J.; Saliba, K. J.; Kirk, K. *Antimicrob. Agents Chemother.* **2004**, *48*, 3241.
22. Ziegler, H. L.; Hansen, H. S.; Staerk, D.; Christensen, S. B.; Hagerstrand, H.; Jaroszewski, J. W. *Antimicrob. Agents Chemother.* **2004**, *48*, 4067.
23. Lolli, G.; Lowe, E. D.; Brown, N. R.; Johnson, L. N. *Structure* **2004**, 2067.
24. Woodard, C. L.; Li, Z.; Kathcart, A. K.; Terrell, J.; Gerena, L.; Lopez-Sanchez, M.; Kyle, D. E.; Bhattacharjee, A. K.; Nichols, D. A.; Ellis, W.; Prigge, S. T.; Geyer, J. A.; Waters, N. C. *J. Med. Chem.* **2003**, *46*, 3877.
25. Woodard, C. L.; Keenan, S. M.; Gerena, L.; Welsh, W. J.; Geyer, J. A.; Waters, N. C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4961.
26. Waters, N. C.; Woodard, C. L.; Prigge, S. T. *Mol. Biochem. Parasitol.* **2000**, *107*, 45.
27. Holton, S.; Merckx, A.; Burgess, D.; Deorig, C.; Noble, M. E.; Endicott, J. *Structure* **2003**, *11*, 1139.
28. Gutteridge, C. E.; Vo, J. V.; Tillett, C. B.; Vigilante, J. A.; Dettmer, J. R.; Patterson, S. L.; Werbovetz, K. A.; Capers, J.; Nichols, D. A.; Bhattacharjee, A. K.; Gerena, L. *Med. Chem.* **2007**, *3*, 115.