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Brief Article

Design, Synthesis, and Development of Novel Guaianolide-Endoperoxides as Potential Antimalarial Agents

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Design and synthesis of a guaianolide-endoperoxide (thaperoxide) **3** was pursued as a new antimalarial lead which was found to be noncytotoxic as compared to the natural product lead thapsigargin **2**. Several analogues of **3** were successfully synthesized and found to be comparable to derivatives of artemisinin **1** in in vitro antimalarial assay. Among the synthesized compounds, **22** showed excellent in vitro potency against the cultured parasites (W2 IC_{50} = 13 nM) without apparent cytotoxicity. Furthermore, SAR trends in thaperoxide analogues are presented and explained with the help of docking studies in the homology model of PfSERCA(PfATP6).

Introduction

Malaria remains a major cause of morbidity and mortality worldwide, with about one million deaths annually.[1] The World Health Organization (WHO) estimates that half of the world's population is at risk of malaria, with the most vulnerable populations including children under the age of five and pregnant women in developing countries.[2,3] At present, with no available vaccine, the prevention and treatment of malaria is increasingly difficult due to the growing resistance of *Plasmodium falciparum* to most available antimalarial drugs.[4,5] Currently, the sesquiterpene lactone artemisinin **1** and its derivatives (collectively called artemisinins) are the only class of compounds consistently effective against multidrug resistant strains of *P. falciparum*. Artemisinins are now routinely recommended as first-line therapy for uncomplicated (as part of artemisinin-based combination therapy)[6] and severe *P. falciparum* malaria. Interestingly, artemisinins also display a wide variety of biological activities, including cytotoxic, antibacterial, and antifungal activities.[7−11] Extensive efforts have been made so far for chemical modifications of artemisinin to improve its therapeutic profile and to reveal its mechanism of action.[12−15] Considering the great therapeutic importance of artemisinins, it is an appropriate strategy to search for novel antimalarials with similar molecular scaffolds to add to our antimalarial armamentarium. The natural product sesquiterpene thapsigargin **2** caught our attention in this regard as a potential lead compound for drug discovery.

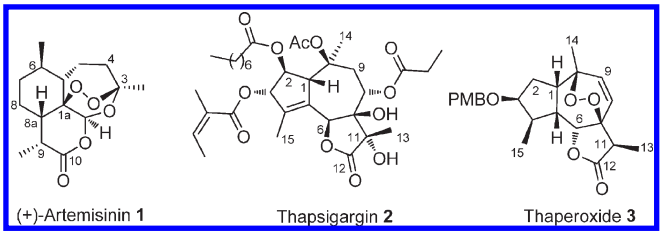

Figure 1. Structures of artemisinin, thapsigargin, and guaianolide-endoperoxide (thaperoxide).

Thapsigargin **2** (see Figure 1) belongs to the guaianolide class of sesquiterpenes and was extensively studied as a highly specific, potent irreversible inhibitor of SERCA[a] (sarco/endoplasmic reticulum Ca^{2+}-dependent ATPase) in the search for promising new apoptotic agents.[16−20] Interestingly, **2** also showed moderate antimalarial activity with a newly proposed mechanism of action inhibiting PfATP6, the *P. falciparum* homologue of SERCA.[21] During our synthetic studies on the synthesis of **2**, we designed a new guaianolide-endoperoxide **3** (named thaperoxide) as a precursor for its total synthesis (Scheme 1). A closer observation of **3** indicates that it has striking structural similarities with both **1** and **2**. On the basis of our computational model,[22] we have found that **3** may obviate the parasite cell permeability problem associated with **2**, and also it can be a valuable probe to evaluate the newly proposed mechanism of action of **1**.[21] Furthermore, we found relatively few guaianolide-peroxides in the literature with the unstable 1α,4α-peroxy-guaianolide scaffold.[23−25] Our search for guaianolides and guaianes revealed that some of them have good antiparasitic activity.[26] These observations including our long-standing efforts to understand the mode of action of **1** prompted us to develop an efficient synthetic strategy to **3**

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[a] Abbreviations: SERCA, sarco/endoplasmic reticulum Ca^{2+}-dependent ATPase; PfSERCA, *P. falciparum* homologue of SERCA; RCM, ring closing metathesis; NaHMDS, sodium bis(trimethylsilyl)amide; Py, pyridine; NOESY, nuclear Overhauser enhancement spectroscopy; DBU, 1,8-diazabicycloundec-7-ene; DDQ, 2,3-dichloro-5,6-dicyanobenzoquinone; PMB, *p*-methoxybenzyl; DIBAL-H, diisobutylaluminum hydride; RBC, red blood cell; SD, standard deviation; VERO, monkey kidney fibroblasts.

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to evaluate its antimalarial activity and cytotoxicity, if present. We planned to synthesize derivatives of **3** in order to derive structure−activity relationships (SAR) of this series of new compounds. As a preliminary study, we compared obtained SAR results with our large pool of data generated from derivatives of **1**[27] and also performed docking studies with the homology model PfSERCA.

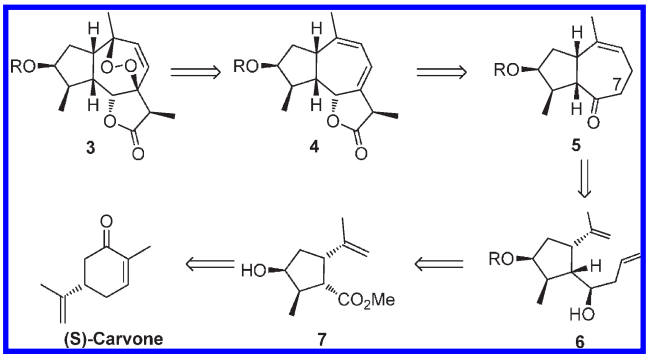
Chemistry

As shown in Scheme 1, we envisioned a stereoselective [4 + 2] cycloaddition of singlet oxygen on diene **4** for introduction of the endoperoxide in **3**. The synthesis of the key intermediate **4** was then anticipated from a bicyclic ketone **5** by homologation at C7 followed by lactonization, which in turn can be obtained from bis-olefin **6** using ring closing metathesis (RCM). A straightforward synthesis was planned for **6** from the known compound **7**.

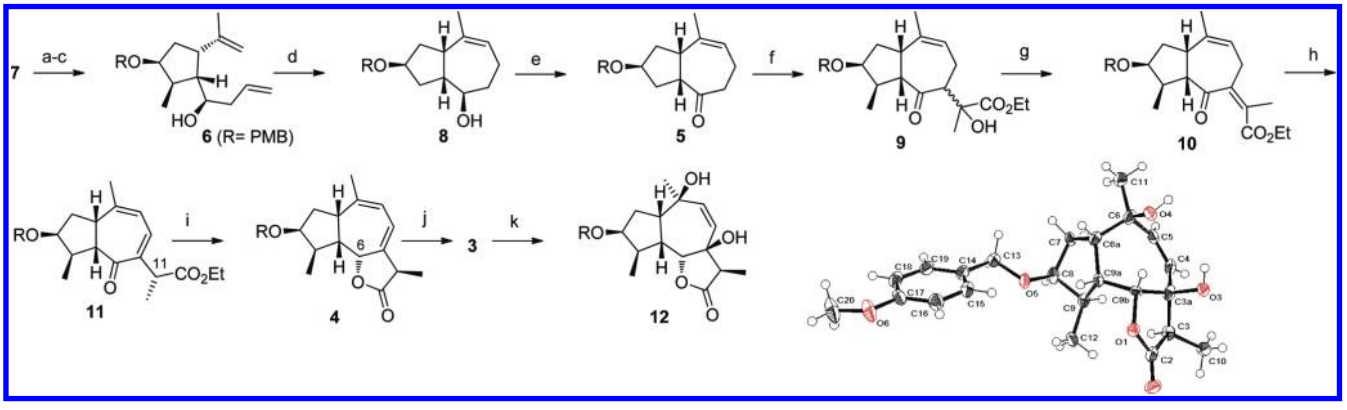
Accordingly, the known ester **7** was obtained from (*S*)-carvone[28] and transformed into RCM precursor **6** in four steps: (i) 3-OH protection, (ii) ester reduction, (iii) oxidation, and (iv) homoallyl Grignard addition (Scheme 2). As predicted by the Felkin−Ahn model, the addition of Grignard nucleophile occurred from less hindered *Si*-face of corresponding aldehyde intermediate to provide **6** exclusively.[29] The proposed RCM of **6** cleanly furnished bicyclic intermediate **8** in the presence of 10 mol % of Grubbs' II catalyst in dichloromethane in high yield. As mentioned in the retrosynthetic analysis, introduction of the lactone ring on the bicyclic system of **8** required C6-OH oxidation for the C7-homologation by selective kinetic enolization. Accordingly, **8** was subjected to Dess−Martin periodinane oxidation to yield ketone **5** in 84% yield. Our initial efforts to homologate the C-7 position of **5** via enolization, followed by treatment with various electrophiles, led to tedious synthetic problems.[30] After several attempts, a facile route was then identified using ethyl pyruvate as a reactive electrophile for aldol reaction. Thus **5** was subjected to kinetic enolization using NaHMDS at −78 °C, and subsequent treatment with ethyl pyruvate gave **9** as a mixture of aldol products in 86% yield. Without further separation, the aldol mixture **9** was then subjected to dehydration using the Martin sulfurine, leading to exclusive *Z*-olefin **10** as confirmed by a 2D NOESY experiment.[31] Subsequent olefin isomerization using DBU led to the required conjugated diene **11** as a single diastereomer as anticipated. The newly generated methyl stereo center of **11** was confirmed as 11*R* at a later stage. Further efforts to form the lactone ring required a straightforward hydride reduction of ketone in **11**, followed by lactonization, to obtain **4**. Sodium borohydride mediated selective reduction of **11** produced a corresponding secondary alcohol, which underwent in situ lactonization, yielding lactone **4** in one pot with a 6*R* configuration. The obtained diene intermediate **4** was then subjected to a final, crucial [4 + 2] cycloaddition using singlet O_2 (generated in situ in the presence of the photosensitizer methylene blue) to furnish the single diastereomeric *endo* adduct **3** in 82% yield.[32] The structure of **3** was unambiguously deduced from single crystal X-ray diffraction studies of the corresponding diol derivative **12**,[33] which was obtained by catalytic hydrogenation of **3** without affecting the C7−C8 double bond. Hence it was clearly evident from the X-ray studies that the A−B−C rings are *cis*-fused and that the C11-methyl group is in the β-configuration.

With the target compound **3** in hand, we evaluated it for antimalarial activity against the chloroquine-resistant (W2) *P. falciparum* strain. To our delight, the thaperoxide **3** showed good antimalarial activity with at least 12-fold better activity (See Table 1) than the corresponding guaianolide natural product thapsigargin **2**. The superior activity of thaperoxide may be due to its greater parasite cell permeability than **2**. Additionally, unlike thapsigargin **2**, thaperoxide **3** did not show any cytotoxicity at 5 μg/mL concentration. Encouraged by these results, we planned the synthesis of various analogues of **3** based on our previous experience with sesquiterpene

Scheme 1. Retrosynthetic Analysis of Guaianolide-Endoperoxide



Scheme 2. Stereoselective Synthesis of Thaperoxide **3**[a]



[a] Reagents and conditions: (a) *p*-methoxybenzyl trichloroacetimidate, $La(OTf)_3$, 0 °C; (b) LAH, Et_2O, rt; (c) DMP, DCM, 0 °C to rt, 3-butenylmagnesium bromide, THF, −78 °C; (d) Grubbs II catalyst, DCM, rt; (e) DMP, DCM, rt; (f) NaHMDS, ethyl pyruvate, THF, −78 °C; (g) Martin reagent, $CHCl_3$, reflux; (h) DBU, DME, 0 °C; (i) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, THF, 0 °C; (j) methylene blue, O_2, 0 °C, DCM; (k) 10% Pd/C, H_2, $CHCl_3$, rt. ORTEP representation of diol **12**.

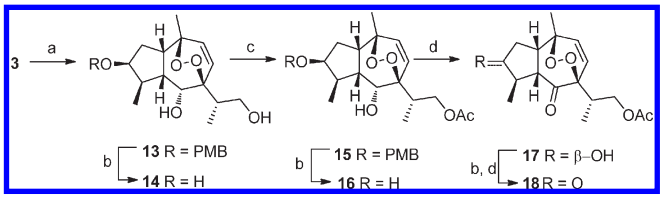
lactones. In the first series, the *seco* analogues **13**−**18** were synthesized from **3** (Scheme 3). We found $LiBH_4$ to be a mild reductant to open the lactone ring in **3** without affecting endoperoxide functionality, furnishing diol **13** in a 94% yield. Selective primary *O*-acetylation of **13** provided **15**, which was oxidized with the Dess−Martin reagent to give the ketone **17**. Meanwhile, PMB-deprotection of **13**, **15**, and **17**, using DDQ, provided the corresponding alcohols **14**, **16**, and **18**, respectively. In the second series of analogue synthesis, we pursued partial reduction of the lactone in **3** to obtain lactol derivatives and deoxo derivatives (Scheme 4).[34] To synthesize the tetracyclic analogues **21**−**24**, the lactone **3** was converted to lactol **20** by treatment with DIBAL-H at −78 °C. The ether derivatives **21**−**22** were obtained directly by acid-catalyzed anomeric etherification, and deoxo analogues **23**−**24** were obtained using triethylsilyl hydride promoted reduction of lactol **20**. Olefin-reduced congeners **28**−**31** were prepared from **25**, which was obtained by diimide reduction of **3**.[35] Thus lactone reduction of **3** or **25** yielded lactol **20** or **28**, followed by further reduction with triethylsilyl hydride, furnished deoxoanalogues **23**, **24**, or **31**. Similar to lactol **20**, lactol **28** was also converted into corresponding ether derivatives **29**−**30**. These analogues were intended to mimic arteether and deoxoartemisinin compounds.

Table 1. Antimalarial Activity against W2 Clones of *P. falciparum*

compd no.	IC_{50} (μM) ± SD[b]	compd no.	IC_{50} (μM) ± SD[b]
3	0.29 ± 0.01[a]	**23**	0.23 ± 0.09
13	9.11 ± 0.21	**24**	0.57 ± 0.04
14	NA	**25**	0.37 ± 0.00[a]
15	NA	**26**	0.61 ± 0.03
16	NA	**27**	0.27 ± 0.02
17	NA	**28**	NA
18	4.60 ± 0.10	**29**	3.40 ± 0.87
19	1.05 ± 0.00	**30**	**0.024** ± 0.02[a]
20	1.04 ± 0.01	**31**	7.50 ± 0.04
21	0.62 ± 0.02[a]	**1** (artemisinin)	0.005 ± 0.00
22	**0.013** ± 0.00[a]	**2** (thapsigargin)	9.92 ± 0.10[c]

[a] These compounds did not show cytotoxicity against VERO (monkey kidney fibroblasts) cells at the concentration greater than 5000 ng/mL.[36] [b] SD = standard deviation. [c] Showed cytotoxicity against VERO cells at 1200 ng/mL; NA = not active up to 10 μM.

Scheme 3. Synthesis of Thaperoxide Analogues without the Lactone Moiety[a]



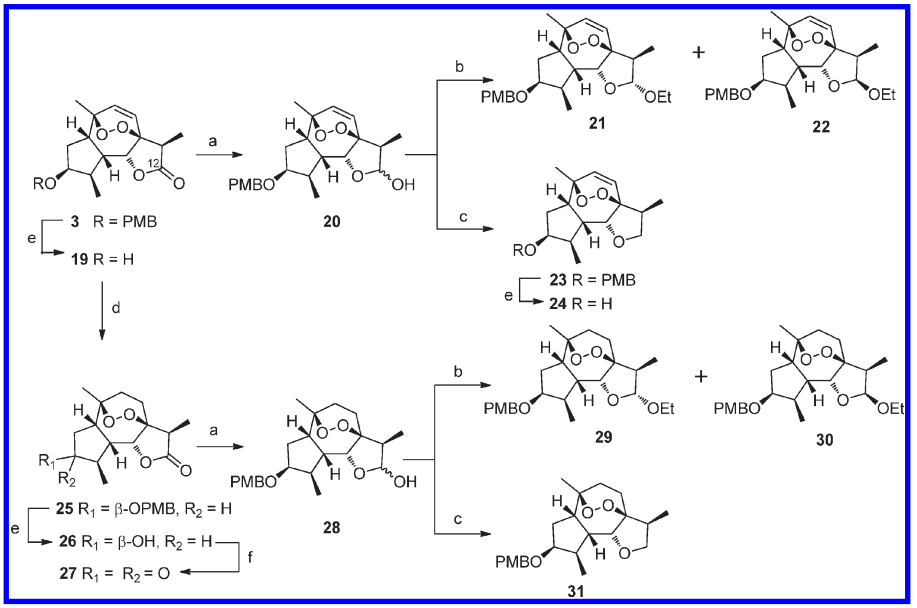
[a] Reagents and conditions: (a) $LiBH_4$, Et_2O, rt; (b) DDQ, DCM, H_2O, rt; (c) Ac_2O, Py, DMAP, DCM, 0 °C to rt; (d) DMP, DCM, 0 °C to rt.

Results and Discussions

The antimalarial activity of thaperoxide and its analogues were determined against the chloroquine resistant W2 strain of *P. falciparum* (Table 1). Thaperoxide **3** displayed submicromolar antimalarial activity and was 12-fold more potent than thapsigargin **2**.

Modifications in the lactone ring had direct effects on the activity and SAR, suggesting that the lactone moiety is essential for antimalarial activity in this class of compounds. Compounds lacking the lactone moiety, such as **13** and **18**, displayed a ∼31- and ∼16-fold decrease in the antimalarial activity of the parent compound **3**, whereas compounds **14**−**17** were inactive at 10 μM. *Seco* analogues of thaperoxide showed only marginal antimalarial activity. Corresponding artemisinin derivatives had variable antimalarial activity. A few *seco* artemisinin analogues reported in the literature were more potent than artemisinin, while most other derivatives showed weak in vitro antimalarial potency. Other modifications in the lactone ring were performed based on the improved potency and pharmacokinetic properties of artemether and 10-deoxyartemisinin derivatives.[34,37,38] Among lactol derivatives, acetal **22**, with a β configuration at C-12, improved antimalarial activity by ∼22 fold. Also, the β-isomer **22** was much superior in potency (∼48 fold) compared to the

Scheme 4. Synthesis of Acetal, Deoxo Derivatives of Thaperoxide[a]



[a] Reagents and conditions: (a) DIBAL-H, DCM, −78 °C; (b) $BF_3 \cdot Et_2O$, benzene, EtOH, 45 °C; (c) $BF_3 \cdot Et_2O$, Et_3SiH, DCM, −78 °C; (d) $KO_2CN{=}NCO_2K$, HOAc, Py, MeOH, DCM, 0 °C to rt; (e) DDQ, DCM, H_2O, rt; (f) DMP, DCM, 0 °C to rt.

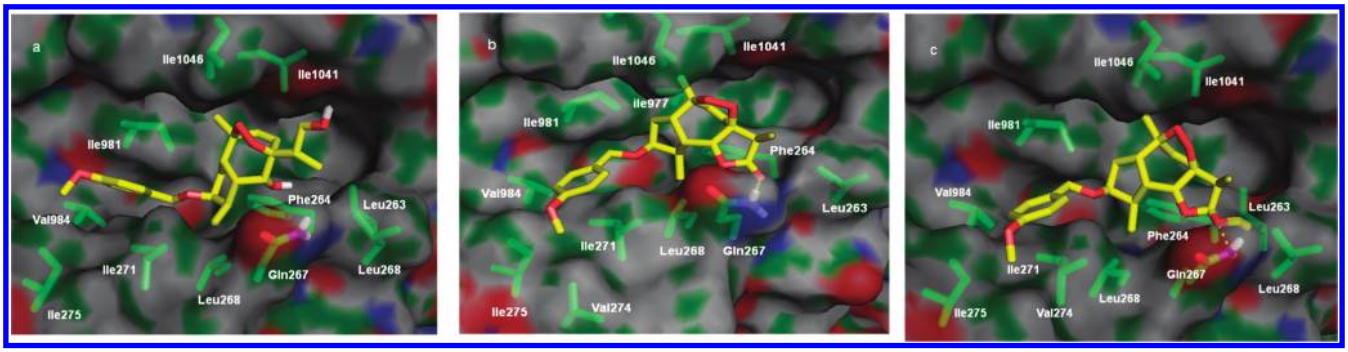


Figure 2. Docking pose of (a) **13**, (b) **3**, (c) **22** in the homology model of PfSERCA. Only side chain atoms of key residues of the active site are shown for clarity. Figures were created using PyMOL1.1 (The PyMOL Molecular Graphics System, version 1.1, Schrodinger, LLC).

corresponding α-isomer **21**. The same activity trend was observed with C7−C8 hydrogenation congeners **29** and **30**. The higher potency of the β-isomer might be attributed to a favorable van der Walls interaction at C-12 for **22** or **30** with corresponding residues of the putative receptor (discussed below).

Modification in the cycloheptane ring, with complete saturation of C7−C8, was expected to improve antimalarial activity by obviating possible peroxide−diepoxidation rearrangement.[39] However, saturation in the cycloheptane ring was found to be unfavorable for antimalarial activity in the thaperoxide series. Saturated analogues (**25**, **28**−**31**) were less active than the corresponding unsaturated analogues (**3**, **20**−**23**). For instance, saturated thaperoxide **25** was at least 1.3-fold less potent than its unsaturated analogues. A similar trend was observed for lactols **20** and **28**, the latter was inactive at the maximum tested concentration (10 μM). Saturated deoxo analogue **31** was minimum 33-fold less potent than the corresponding unsaturated deoxy derivative **23**. However, to our surprise, alcohol **26** in the C7−C8 saturated series was 1.7-fold more potent than the unsaturated alcohol **19**. Finally, the effect of the C3-OPMB protecting group was observed, as the C3-OH derivatives obtained by deprotection were inferior in activity compared to compounds with the PMB group. For example, compound **19** showed a 3.6-fold drop in potency compared to that of **3** upon removal of the PMB group. Similarly, the deprotected analogue of **25** (compound **26**) showed about half the potency of the parent compound.

We analyzed our SAR results based on interaction with the proposed target PfATP6 by construction of a homology model of PfATP6. This model was constructed using the crystal structure of human SERCA (PDB CODE: 2AGV, full details in Supporting Information). Docking of compounds **3**, **13**, and **22** was carried out in the active site of SERCA using the GOLD docking program (Figure 2).[40] Top ranked poses of these compounds are shown in Figure 2. In the model, these compounds maintained the same orientation of the tricyclic scaffold as seen in the X-ray structure of thapsigargin in the complex with mammalian SERCA. The key determinants of thaperoxide binding to PfATP6 involved hydrophobic interactions with the active site residues of SERCA, with the exposure of the peroxide bridge to the solvent. Careful analysis of binding site interactions further revealed three major structural features important for activity within this series of compounds, namely the 5−7−5 tricyclic system, the lactone ring, and the hydrophobic substituent at the C-3 position. The tricyclic scaffold of compound **3** (Figure 2b) occupied the binding pocket around Phe264, similar to the binding of thapsigargin in the crystal structure of human SERCA. This interaction was facilitated by the complementary shape of the sesquiterpene lactone to the PfATP6 binding pocket. Also, the lactone carbonyl was found to form a hydrogen bond (2.4 Å) with the side chain amide of Gln267. Furthermore, it was noted that the PMB group at the C-3 position contributed to the binding of thaperoxide analogues through hydrophobic interactions with Ile271, Val274, Ile275, and Ile981 of the predicted PfATP6 active site. The drastic reduction in the activity of **13** (seco analogue) compared to **3** can result from the loss of the complementary shape required for the proper hydrophobic interaction around Phe264 placing α-hydroxy group on C-6 in the hydrophobic environment (Figure 2a). The most active compound in this series, **22**, showed a similar binding mode to compound **3**. The improved activity of **22** might be attributed to additional hydrophobic interactions between the ethoxy group on C-12 with Leu263 as well as the formation of a strong hydrogen bond with Gln267, which is within 2.0 Å of **22** (see Figure 2c). These observations showed that docking simulations were able to support experimental results to some extent and provided valuable insights about the structure−activity relationship of this novel series of peroxy analogues. Although convincing, these results left certain ambiguities about *Pf*ATP6 as a possible target for specifically artemisinin. We noticed that no biochemical evidence of mobilization of intracellular calcium into *P. falciparum* resultant from binding of artemisinin to *Pf*ATP6. Since the antagonistic relationship between **1** and **2** is reported in an in vitro study supporting the inhibition of pfATP6,[21] we consider that the most active analogues of thaperoxide (**22** or **30**, equipotent to artemisinin **1**) will provide more inputs in similar experiments. Further studies are in progress in this regard and will be disclosed in due course.

Conclusion

In summary, we have designed and synthesized new guaianolide-endoperoxides as potential antimalarial agents. In this search, we identified thaperoxide (**3**)/analogues as simplified analogues of thapsigargin **2** in terms of structural complexity to reduce the synthetic challenge and cost. Most importantly, installation of crucial peroxide pharmacophore on the backbone of guaianolide **3** improved its antimalarial potency and precluded its cytotoxicity compared to **2**. Various structural features of **3**, such as lactone, guaianolide tricyclic systems, and peroxide moiety, are found to be critical to retain and to improve its antimalarial activity. In addition, a homology model of PfATP6 revealed the importance of C3-hydrophobic (OPMB) and C12-β substituents in **3** crucial for the potency in this series. These detailed SAR studies provided important information for future structural modifications. Further studies

in this area are warranted and are currently underway in our laboratory.

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Supporting Information Available: Synthesis methods and characterization data of all compounds along with details of modeling and biological assay procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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