JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

8,8-Dialkyldihydroberberines with Potent Antiprotozoal Activity

Molla Endeshaw,[†] Xiaohua Zhu,[†] Shanshan He,[†] Trupti Pandharkar,[†] Emily Cason,[‡] Kiran V. Mahasenan,[†] Hitesh Agarwal,[†] Chenglong Li,[†] Manoj Munde,[§] W. David Wilson,[§] Mark Bahar,[†] Raymond W. Doskotch,[†] A. Douglas Kinghorn,[†] Marcel Kaiser,[⊥,∥] Reto Brun,[⊥,∥] Mark E. Drew,[†,‡] and Karl A. Werbovetz*[,†]

[†]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, United States

[‡]Department of Microbial Infection and Immunity, College of Medicine, The Ohio State University, Columbus, Ohio 43210, United States

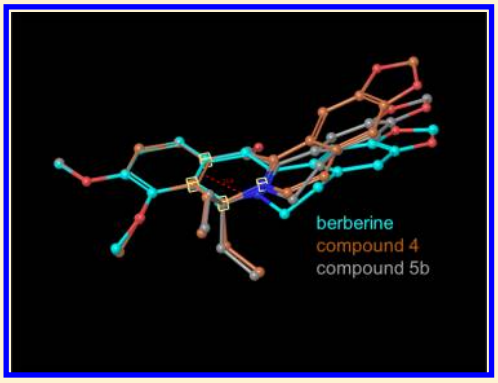
[§]Department of Chemistry, Georgia State University, Atlanta, Georgia 30302, United States

[⊥]Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, CH-4002 Basel, Switzerland

[∥]University of Basel, CH-4002 Basel, Switzerland

Supporting Information

ABSTRACT: Semisynthetic 8,8-dialkyldihydroberberines (8,8-DDBs) were found to possess mid- to low-nanomolar potency against *Plasmodium falciparum* blood-stage parasites, *Leishmania donovani* intracellular amastigotes, and *Trypanosoma brucei brucei* bloodstream forms. For example, 8,8-diethyldihydroberberine chloride (**5b**) exhibited in vitro IC_{50} values of 77, 100, and 5.3 nM against these three parasites, respectively. In turn, two 8,8-dialkylcanadines, obtained by reduction of the corresponding 8,8-DDBs, were much less potent against these parasites in vitro. While the natural product berberine is a weak DNA binder, the 8,8-DDBs displayed no affinity for DNA, as assessed by changes in the melting temperature of poly(dA·dT) DNA. Selected 8,8-DDBs showed efficacy in mouse models of visceral leishmaniasis and African trypanosomiasis, with 8,8-dimethyldihydroberberine chloride (**5a**) reducing liver parasitemia by 46% in *L. donovani*-infected BALB/c mice when given at an intraperitoneal dose of 10 mg/kg/day for five days. The 8,8-DDBs may thus serve as leads for discovering new antimalarial, antileishmanial, and antitrypanosomal drug candidates.



Although berberine (**1**) was first described in the 19th century, this compound continues to be investigated for its biological properties, as accounts of the anticancer,[1] antidiabetic,[2] and antiviral[3] properties of this plant quaternary alkaloid were recently published. Berberine also possesses activity against protozoans that cause malaria,[4,5] leishmaniasis,[6,7] and African trypanosomiasis.[8] The clinically used drugs available to treat these parasitic infections display one or more limitations, including toxicity, expense, the need for parenteral administration, and decreased efficacy due to resistance. Berberine has poor bioavailability,[9] requiring large oral doses to produce a pharmacological effect,[6,10] and the in vitro potency of berberine is lower than that of standard antiprotozoal drugs.[5,8] Thus, improvements in the potency and physicochemical properties of berberine are required before related compounds can be considered as candidates against malaria, leishmaniasis, and/or African trypanosomiasis.

Our previous evaluation of berberine analogues synthesized many years earlier[11] resulted in the identification of a sample with exceptional in vitro antiprotozoal potency. Analysis of this derivative, initially thought to be 8,8-diethyldihydroberberine (**5b**), revealed it to be a mixture of compounds. Resynthesis based on the original procedure[11] resulted in the isolation and structural characterization of a novel compound, 5,6-didehydro-8,8-diethyl-13-oxodihydroberberine (**4**), where oxidation had taken place at C-13 and between C-5 and C-6 of the protoberberine core.[12] Compound **4** retained the potent antiparasitic activity of the initial sample and displayed efficacy in a murine visceral leishmaniasis model.[12] However, **4** could be given to mice only at 1 mg/kg/day when administered ip. Thus, a critical step in the further progression of molecules related to **4** as antiparasitic candidates is to decrease toxicity while retaining antiparasitic efficacy.

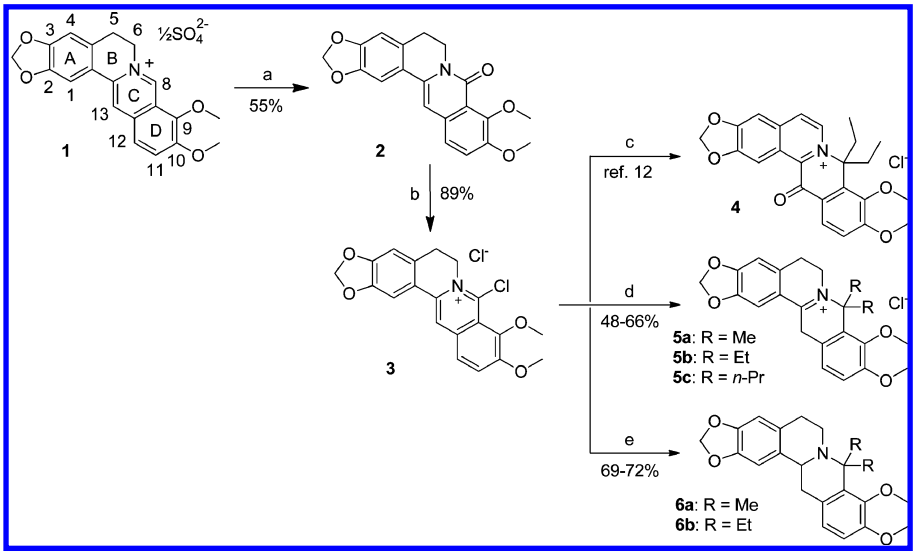
Cheng et al. reported the 8,8-dialkyldihydroberberines (8,8-DDBs), **5a**–**5c**, and investigated their antidiabetic activity.[13] Compound **5a** promoted glucose uptake and AMPK phosphorylation in L6 myoblasts and reduced glucose levels

Special Issue: Special Issue in Honor of Lester A. Mitscher

Received: September 17, 2012

dx.doi.org/10.1021/np300638f | *J. Nat. Prod.* XXXX, XXX, XXX–XXX

Scheme 1[a]



[a]Reagents and conditions: (a) aq NaOH or KOH, reflux; (b) $POCl_3$, reflux; (c) 1-EtMgBr, Et_2O, reflux; 2-H_2SO_4, ice, then HCl (g); (d) 1-MeMgBr (3 M in Et_2O), EtMgBr (3 M in Et_2O), or *n*-PrMgBr (2 M in THF), Et_2O, reflux; 2-NH_4OH, then HCl(g) in EtOAc, rt; (e) 1-MeMgBr or EtMgBr (3 M in Et_2O), Et_2O, reflux; 2-NH_4OH; 3-$NaBH_4$, MeOH.

Table 1. In Vitro Activity (nM) of Berberine and 8,8-DDBs[a]

compound	IC_{50} vs *Pf*[b]	IC_{50} vs *Ld*[c]	IC_{50} vs *Tbb*[d]	IC_{50} vs Vero cells
1	800 ± 310[e]	17 000 ± 3000	1100 ± 100[e]	>200 000
4	20 ± 4	290 ± 40	2.0 ± 0.0	39 000 ± 4000
5a	63 ± 10	200 ± 50	4600 ± 400	8800 ± 1800
5b	77 ± 34	100 ± 10	5.3 ± 0.9	18 000 ± 1000
5c	30 ± 1	550 ± 60	53 ± 9	19 000 ± 1000
6a	7300 ± 600	7300 ± 3100[f]	52 000 ± 4000	ND[g]
6b	1700 ± 0	8900 ± 300[f]	230 ± 10	ND
CQ[h]	8.3 ± 1.7	ND	ND	ND
AmB[i]	ND	46 ± 10	ND	ND
Sur[j]	ND	ND	130 ± 10	ND
Podo[k]	ND	ND	ND	17 ± 1

[a]Mean ± standard error ($n \geq 3$ unless noted otherwise). [b]*P. falciparum*. [c]*L. donovani*. [d]*T. brucei brucei*. [e]Mean ± range ($n = 2$), results taken from ref 12. [f]Mean ± range ($n = 2$). [g]Not determined. [h]Chloroquine. [i]Amphotericin B. [j]Suramin. [k]Podophyllotoxin.

in diabetic mice. Cheng et al. also reported that **5a** has an oral bioavailability of 10%, much higher than that of berberine or dihydroberberine. In the present study, the 8,8-DDBs **5a**–**5c** and the canadine derivatives **6a** and **6b** were prepared semisynthetically and evaluated for their in vitro antiprotozoal activity. The in vivo antileishmanial and antitrypanosomal efficacy of the compounds with the highest in vitro potency were also evaluated.

RESULTS AND DISCUSSION

Compounds **5a**–**5c** were synthesized from **1** through the intermediates 8-oxoberberine (**2**) and 8-chloroberberine (**3**) in a similar manner to **4**, using slight modifications of literature procedures (Scheme 1).[11−13] A suspension of **3** in diethyl ether was treated with methyl, ethyl, and *n*-propyl Grignard reagents followed by ammonium hydroxide workup[13] to provide the corresponding 8,8-DDB-free bases. The acidic workup used previously likely leads to oxidation of the 8,8-DDB product, as observed in the synthesis of **4**.[12] The 8,8-DDB-free bases were unstable and were thus converted into their stable salts in 48–66% yield by bubbling HCl gas through a solution of the free bases in EtOAc. Canadine derivatives **6a** and **6b** were synthesized by borohydride-mediated reduction of the 8,8-DDB-free bases obtained after dialkylation of **3** in good overall yield (69–72% from **3**). ^1H and ^{13}C NMR spectra were consistent with those previously reported for **5a**–**5c**,[13] and HPLC analysis indicated that target compounds **5a**–**5c**, **6a**, and **6b** were obtained in ≥95% purity. Detailed methods and characterizations for the target compounds are provided in the Supporting Information.

In vitro biological activities of the 8,8-DDBs **4** and **5a**–**5c** and the 8,8-dialkylcanadines **6a** and **6b** together with those of **1** are given in Table 1. Against erythrocyte-stage *P. falciparum* 3D7 parasites, compounds **4** and **5c** displayed the best efficacy, with the range of potency being less than 4-fold among the 8,8-DDBs. Compounds **4** and **5c** exhibited in vitro antimalarial potency within 4-fold of chloroquine against this susceptible strain. The diethyl compound **5b** was the most potent 8,8-DDB against intracellular *L. donovani*, exhibiting approximately 2-fold lower potency than the antileishmanial drug amphotericin B and 2-fold greater potency than **5a**. In turn, the diethyl derivatives **4** and **5b** were the most potent molecules in this series against bloodstream-form *Trypanosoma brucei brucei*, displaying low nanomolar IC_{50} values and superior in vitro

dx.doi.org/10.1021/np300638f | J. Nat. Prod. XXXX, XXX, XXX−XXX

antitrypanosomal efficacy compared to suramin. The carbonyl-containing compound **4** exhibited 2.5-fold greater potency than **5b**, while the dipropyl and dimethyl derivatives **5c** and **5a** were 27-fold and 2300-fold less potent than **4**, respectively. The 8,8-dialkylcanadines **6a** and **6b** were much less active than the corresponding 8,8-DDBs against all three parasites. As with 8,8-DDBs **5a** and **5b**, dimethyl canadine (**6a**) is far less potent against African trypanosomes than diethyl congener **6b** (230-fold). In general, the in vitro selectivity indexes for the 8,8-DDBs (IC_{50} value vs Vero cells/IC_{50} value vs parasites) were excellent. The selectivity indexes for **4** and **5b** were greater than 100 against *L. donovani* and over 3000 against *T. b. brucei*, while these indexes were greater than 600 for **4** and **5c** against *P. falciparum*.

Given their outstanding in vitro potency, compounds **5a**−**5c** were evaluated for their toxicity to female BALB/c mice to determine an appropriate dosing regimen for in vivo efficacy studies. Compounds **5a**−**5c** displayed no overt toxicity when given ip to mice at 10 mg/kg/day × 5. Administration of **5a** at 10 mg/kg/day × 5 starting at 7 days postinfection by the ip route to female BALB/c mice infected with *L. donovani* parasites resulted in 46% inhibition of liver parasitemia when assessed at 14 days postinfection. Compounds **5b** and **5c** were less effective in this model when given at the same dose and assessed under the same conditions (Table 2).

Table 2. In Vivo Efficacy of 8,8-DDBs in a Murine Model of Visceral Leishmaniasis

compound	dose (mg/kg)	% inhibition of liver parasitemia (mean ± SD, n = 4)
5a	10 × 5 ip	46 ± 3
5b	10 × 5 ip	29 ± 11
5c	10 × 5 ip	32 ± 10
miltefosine	10 × 5 ip	97 ± 2

On the basis of their potency against African trypanosomes in vitro, **4** and **5b** were evaluated for efficacy in female NMRI mice infected with *T. b. rhodesiense* STIB900 parasites. Infected animals were treated with compounds or vehicle daily from day 3 to 6 postinfection; then parasitemia was assessed on day 7 postinfection and twice weekly thereafter. When administered ip at 1 mg/kg/day × 4, compound **4** reduced parasitemia in all infected mice on day 7, but each animal in this group displayed heavy parasitemia on day 10 (Table 3). Treatment with compound **5b** at 10 mg/kg/day × 4 ip produced a more variable response in infected mice, with two animals having cleared parasitemia by day 7 and relapsing as late as day 10 and 18 postinfection. One animal in the group treated with **5b** was found dead on day 7 postinfection; the cause of death is not known. The antitrypanosomal drug pentamidine cures a subset of infected animals in this model when given ip at 20 mg/kg/day × 4.[14]

Table 3. In Vivo Efficacy of 4 and 5b against *T. b. rhodesiense* STIB900

compound	dose (mg/kg)	cured/ infected	day of relapse			
control	untreated	0/3	7[a]	7[a]	7[a]	
4	1 × 4 ip	0/4	7[b]	7[b]	7[b]	7[b]
5b	10 × 4 ip	0/4	18	10	dead on d7	7[b]

[a]Control mice displayed heavy parasitemia on day 7. [b]Mice were weakly positive for trypanosomes on day 7 and heavily positive on day 10.

Since previous studies demonstrated the binding of **1** to DNA,[15,16] we examined the DNA binding properties of the 8,8-DDBs (Table 4). The change in melting temperature (ΔT_m)

Table 4. Increase in Poly(dA·dT) Melting by 1 and 8,8-DDBs

compound	ΔT_m (°C)
1	3.5
4	0.5
5a	0.3
5b	0.5
5c	0.0

caused by **1** was modest, consistent with earlier work.[15] Little to no increase in the ΔT_m of poly(dA)·poly(dT) DNA was observed in the presence of **4** and **5a**−**5c**, suggesting that these compounds are not DNA binders.

Considering the lack of DNA binding of the 8,8-DDBs and their superior antiparasitic activity compared to **1**, we examined the conformations of **1** and 8,8-diethyl derivatives **4** and **5b** by molecular modeling. For the energy-minimized conformations of **1**, **4**, and **5b**, the dimethoxyaryl ("D") ring present in all analogues was superimposed (see Scheme 1 for the labeling and numbering of the protoberberine ring system). A larger dihedral angle between the boxed atoms of the "C" ring was observed for **4** (15.8°) and **5b** (8.5°) compared to **1** (0.1°). Thus, both **4** and **5b** prefer a more bent geometry than **1** due to the loss in aromaticity of ring C.

While **4** displayed exceptional in vitro potency against malaria parasites, *Leishmania*, and African trypanosomes and exhibited efficacy in a mouse model of visceral leishmaniasis, this compound was toxic to mice at relatively low doses.[12] However, removing the C-13 carbonyl and the C-5−C-6 double bond resulted in the 8,8-DDBs **5a**−**5c**, which displayed in vitro antiprotozoal potency comparable to that of **4**. The C-13 carbonyl and the C-5−C-6 double bond present in **4** are thus not absolute requirements for antiparasitic activity. Despite their in vitro potency, moderate to weak in vivo efficacy was observed with 8,8-DDBs in murine models of visceral leishmaniasis and African trypanosomiasis. As mentioned earlier, Cheng et al. reported that the oral bioavailability of **5a** was 10%, superior to that of **1** or dihydroberberine,[13] but there is no further information regarding the pharmacokinetic properties of **5a** or the other 8,8-DDBs examined here. Since a compound's pharmacokinetic profile has a profound impact on its in vivo efficacy, a detailed investigation of such properties for 8,8-DDBs is required to rationalize the apparent disconnect between in vitro and in vivo antiparasitic efficacy.

Compounds **5a**−**5c** were approximately 2- to 4-fold more toxic to Vero cells in vitro compared to **4** (Table 1), but could be administered to BALB/c mice at 10-fold higher doses than **4** by the ip route. The 8,8-DDBs evaluated here may elicit cell type- or organ system-specific toxicity that is not reproduced by assays performed on Vero cells or the peritoneal macrophage host cells we employed. The problem of correlating in vitro and in vivo toxicity data has been noted previously.[17] Identification of the biological targets of the 8,8-DDBs is needed to better characterize the effects of these compounds on both parasites and host cells. Berberine is active against numerous cellular

dx.doi.org/10.1021/np300638f | J. Nat. Prod. XXXX, XXX, XXX−XXX

targets and also binds to DNA.[15,16] Since the 8,8-DDBs did not affect the melting temperature of poly(dA·dT) DNA (Table 4), it is unlikely that these compounds either act on parasites by binding directly to DNA or possess mutagenic effects in cells. The lack of planarity of compounds **4** and **5b** (Figure 1) may

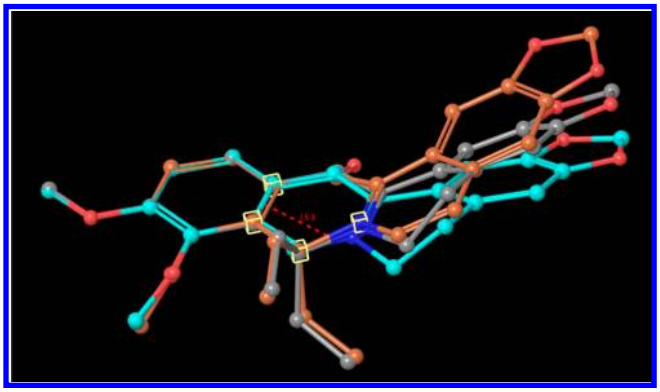


Figure 1. Energy-minimized conformations of **1** (cyan), **4** (coral), and **5b** (gray) superimposed by ring D. Dihedral angles were calculated for the indicated atoms of the "C" ring (yellow boxes). The largest dihedral angle of these three compounds, occurring in **4**, is shown in red.

prohibit the binding of these semisynthetic berberine derivatives to DNA. Berberine[13,18] and the 8,8-DDBs **5a**–**5c**[13] promote the phosphorylation of the metabolic regulatory protein AMP-dependent protein kinase (AMPK). The presence of AMPK subfamily genes was proposed in kinetoplastid parasites,[19] and homologues of AMPK subunits β and γ were partially characterized in *T. brucei*.[20] Further studies are required to formulate a hypothesis concerning the antiparasitic mechanism(s) of action of the 8,8-DDBs. If the antiparasitic target(s) of the 8,8-DDBs is(are) distinct from the mammalian target(s) resulting in toxicity, it may be possible to increase the in vivo selectivity of these compounds while optimizing their efficacy.

Given the shortcomings of the current drugs used against malaria, leishmaniasis, and African trypanosomiasis, a series of compounds capable of providing leads against one or more of these diseases should be of great interest. The 8,8-DDBs possess several favorable attributes as an antiparasitic class. First, they display outstanding in vitro potency and selectivity against the relevant parasites. Second, the 8,8-DDBs reported here can be prepared in three steps from the relatively inexpensive plant alkaloid berberine. Third, they possess activity in murine models of visceral leishmaniasis and African trypanosomiasis, providing proof of concept that 8,8-DDBs display in vivo antiparasitic efficacy. Finally, distinctions in the emerging antiparasitic structure–activity relationship for 8,8-DDBs and 8,8-dialkylcanadines indicate that it should be possible to design related molecules with selectivity for one protozoan compared to another, boding well for the identification of highly specific antiparasitic agents. A broader investigation of the 8,8-DDBs and their analogues is thus warranted in the search for new drug candidates against protozoan parasites.

EXPERIMENTAL SECTION

General Experimental Procedures. All reagents and solvents were from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin-layer chromatography was conducted on precoated TLC plates from E. Merck, and compounds were visualized with UV light. Nuclear magnetic resonance spectra were obtained at 300 or 400 MHz for ^{1}H and 75 or 100 MHz for ^{13}C using instruments from Bruker. Purity of the target compounds was assessed using either a Phenomenex Gemini 5 μm C_{18} Axis packing reversed-phase column (250 × 4.1 mm) or a Merck LichroCART Lichrospher 100 RP-18 10 μm reversed-phase column employing a Hitachi HPLC system (L-2130) and a diode array detector (L-2455) at a constant flow rate of 1 mL/min. Mobile phase A consisted of water containing 0.1% trifluoroacetic acid, while mobile phase B consisted of acetonitrile containing 0.1% trifluoroacetic acid. The gradient employed ran from 0% to 100% mobile phase B over 22 min, then from 100% to 0% mobile phase B over 8 min (total run time of 30 min). Details of the synthetic procedures and compound characterizations are available as Supporting Information.

In Vitro Assays. In vitro antimalarial,[21] antileishmanial,[22] and antitrypanosomal[23] efficacy testing and cytotoxicity evaluation[24] of the compounds was carried out as described previously. The binding of test compounds to poly(dA)·poly(dT) DNA was assessed by a method reported earlier.[25]

Evaluation of in Vivo Efficacy. The efficacy of test and control compounds against *L. donovani* LV82 parasites in female BALB/c mice was examined by methods described previously.[26] Evaluation of compounds for efficacy in female NMRI mice infected with *T. b. rhodesiense* STIB900 parasites was performed according to the procedure outlined by Wenzler et al.[14] in which infected animals were treated with test compounds or vehicle daily from day 3 to 6 postinfection, with the minor modification that parasitemia was recorded by tail blood examination on day 7 postinfection and twice weekly thereafter. The day of relapse was recorded after detection of parasitemia; mice with a heavy parasite load were euthanized. Protocols for in vivo experiments were approved by either the Institutional Animal Care and Use Committee at The Ohio State University (*L. donovani* model) or the veterinary authorities of the Canton Basel-Stadt, Switzerland (*T. b. rhodesiense* model).

Molecular Modeling. Compound coordinates were built using the Maestro v9.1 interface of Schrödinger Suite 2010. After initial geometry cleanup, compounds were subjected to Macromodel energy minimization (Batchmin v9.8). Potentials from OPLS force field 2005 and a constant dielectric of 1.0 were used with water as solvent. A maximum of 500 steps with a convergent threshold of 0.05 rmsd was applied for the PRCG minimization method.

ASSOCIATED CONTENT

Supporting Information

Details of the synthetic procedures and compound characterizations are available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: +1 614 292 5499. Fax: +1 614 292 2435. E-mail: werbovetz.1@osu.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported financially by the Ohio State University Public Health Preparedness for Infectious Diseases program.

DEDICATION

Dedicated to Dr. Lester A. Mitscher, of the University of Kansas, for his pioneering work on the discovery of bioactive natural products and their derivatives.

REFERENCES

(1) Li, J.; Cao, B.; Liu, X.; Fu, X.; Xiong, Z.; Chen, L.; Sartor, O.; Dong, Y.; Zhang, H. *Mol. Cancer Ther.* **2011**, *10*, 1346−1356.
(2) Chueh, W. H.; Lin, J. Y. *J. Agric. Food Chem.* **2011**, *59*, 8021−8027.
(3) Cecil, C. E.; Davis, J. M.; Cech, N. B.; Laster, S. M. *Int. Immunopharmacol.* **2011**, *11*, 1706−1714.
(4) Vennerstrom, J. L.; Klayman, D. L. *J. Med. Chem.* **1988**, *31*, 1084−1087.
(5) Wright, C. W.; Marshall, S. J.; Russell, P. F.; Anderson, M. M.; Phillipson, J. D.; Kirby, G. C.; Warhurst, D. C.; Schiff, P. L. *J. Nat. Prod.* **2000**, *63*, 1638−1640.
(6) Vennerstrom, J. L.; Lovelace, J. K.; Waits, V. B.; Hanson, W. L.; Klayman, D. L. *Antimicrob. Agents Chemother.* **1990**, *34*, 918−921.
(7) Saha, P.; Bhattacharjee, S.; Sarkar, A.; Manna, A.; Majumder, S.; Chatterjee, M. *PLoS One* **2011**, *6*, e18467.
(8) Merschjohann, K.; Sporer, F.; Steverding, D.; Wink, M. *Planta Med.* **2001**, *67*, 623−627.
(9) Qiu, F.; Zhu, Z.; Kang, N.; Piao, S.; Qin, G.; Yao, X. *Drug Metab. Dispos.* **2008**, *36*, 2159−2165.
(10) Zhou, J.; Zhou, S.; Tang, J.; Zhang, K.; Guang, L.; Huang, Y.; Xu, Y.; Ying, Y.; Zhang, L.; Li, D. *Eur. J. Pharmacol.* **2009**, *606*, 262−268.
(11) Moniot, J. L.; Kravetz, T. M.; Abd el Rahman, A. H.; Shamma, M. *J. Pharm. Sci.* **1979**, *68*, 705−708.
(12) Bahar, M.; Deng, Y.; Zhu, X.; He, S.; Pandharkar, T.; Drew, M. E.; Navarro-Vázquez, A.; Anklin, C.; Gil, R. R.; Doskotch, R. W.; Werbovetz, K. A.; Kinghorn, A. D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2606−2610.
(13) Cheng, Z.; Chen, A. F.; Wua, F.; Sheng, L.; Zhang, H. K.; Gu, M.; Li, Y. Y.; Zhang, L. N.; Hu, L. H.; Li, J.-Y.; Li, J. *Bioorg. Med. Chem.* **2010**, *18*, 5915−5924.
(14) Wenzler, T.; Boykin, D. W.; Ismail, M. A.; Hall, J. E.; Tidwell, R. R.; Brun, R. *Antimicrob. Agents Chemother.* **2009**, *53*, 4185−4192.
(15) Mazzini, S.; Bellucci, M. C.; Mondelli, R. *Bioorg. Med. Chem.* **2003**, *11*, 505−514.
(16) Ferraroni, M.; Bazzicalupi, C.; Bilia, A. R.; Gratteri, P. *Chem. Commun.* **2011**, *47*, 4917−4919.
(17) McKim, J. M. J. *Comb. Chem. High Throughput Screening* **2010**, *13*, 188−206.
(18) Cheng, Z.; Pang, T.; Gu, M.; Gao, A. H.; Xie, C. M.; Li, J.-Y.; Nan, F.; Li, J. *Biochim. Biophys. Acta* **2006**, *1760*, 1682−1689.
(19) Parsons, M.; Worthey, E. A.; Ward, P. N.; Mottram, J. C. *BMC Genomics* **2005**, *6*, 127.
(20) Clemmens, C. S.; Morris, M. T.; Lyda, T. A.; Acosta-Serrano, A.; Morris, J. C. *Exp. Parasitol.* **2009**, *123*, 250−257.
(21) Liu, J.; Gluzman, I. Y.; Drew, M. E.; Goldberg, D. E. *J. Biol. Chem.* **2005**, *280*, 1432−1437.
(22) Zhu, X.; Pandharkar, T.; Werbovetz, K. *Antimicrob. Agents Chemother.* **2012**, *56*, 1182−1189.
(23) Werbovetz, K. A.; Sackett, D. L.; Delfin, D.; Bhattacharya, G.; Salem, M.; Obrzut, T.; Rattendi, D.; Bacchi, C. *Mol. Pharmacol.* **2003**, *64*, 1325−1333.
(24) Salem, M. M.; Werbovetz, K. A. *J. Nat. Prod.* **2005**, *68*, 108−111.
(25) Wilson, W. D.; Tanious, F. A.; Fernandez-Saiz, M.; Rigl, C. T. *Methods Mol. Biol.* **1997**, *90*, 219−240.
(26) Delfín, D. A.; Morgan, R. E.; Zhu, X.; Werbovetz, K. A. *Bioorg. Med. Chem.* **2009**, *17*, 820−829.

dx.doi.org/10.1021/np300638f | *J. Nat. Prod.* XXXX, XXX, XXX−XXX