Med Chem Res (2011) 20:401–407
DOI 10.1007/s00044-010-9325-2

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of a novel anti-malarial lead

Nicholas L. Hammond · Seoung-ryoung Choi · Paulo Carvalho · Hua Liu · Shabana Khan · Mitchell A. Avery

Received: 23 June 2009 / Accepted: 12 February 2010 / Published online: 20 July 2010

Abstract Malaria is re-emerging in many tropical areas of the world and is often fatal due to drug resistance, leading to about a million deaths each year. Multiple drug resistance has required new efforts in drug discovery and development. Thus, the search for new drugs operating by novel mechanisms of action is receiving increased attention. Herein we report the synthesis and biological evaluation of a novel anti-malarial with micromolar activity against resistant strains of the parasite.

Keywords Malaria · Plasmodium · Diamine · Imidazole

Introduction

Parasitic infections are a major cause of mortality in third world nations, with malaria being one of the most severe tropical diseases. Malaria, caused by protozoa from the genus *Plasmodium*, is possibly the most serious parasitic disease encounters due to prevalence, virulence, and drug resistance. The World Health Organization (WHO) has designated malaria as the first priority among tropical diseases because of its reemergence as the biggest infectious killer. Recent estimates affirm that malaria may affect more than 2.4 billion people in more than 100 countries, accounting for about 40% of the world's population (Ridley, 2002). Annually, between 300 and 500 million people will be afflicted with malaria and result in 2–3 million deaths, with 3,000 children under the age of 5 dying each day (Butcher *et al.*, 2000). Malaria is ranked third, behind pneumococcal infections and tuberculosis, for major infectious diseases resulting in death.

Only one synthetic anti-malarial drug (mefloquine, **1**) has been discovered in the last 30 years (Vangapandu *et al.*, 2007). Artemisinin (**2**) was also discovered during this period but has been known to have medicinal properties for more than 2,000 years. The recent and rapid increase of drug resistance of *Plasmodium falciparum* (*P. falciparum*) to chloroquine (**3**) has absolutely necessitated the discovery of novel anti-malarial agent. There are four classes of malarial drugs which target the parasite at different stages of its life cycle. Blood schizontocides target the blood phase of the parasite life cycle and include such drugs as chloroquine, quinine, mefloquine, and artemisinin. Tissue schizontocides, such as primaquine, act on the parasite while in the liver tissue. The gametocytocides act on the sexual forms of the parasite in the blood phase to prevent transmission to mosquitoes and include chloroquine, quinine, and primaquine. Finally, the sporontocides, such as tafenoquine, prevent the development of the oocyt in the mosquito thereby preventing transmission.

There are several proposed mechanisms of action for the 4-aminoquinolones including intercalation with the DNA of the malaria parasite, impairment of lysosome function, inhibition of heme-dependent protein synthesis, and inhibition of heme polymerization (Meshnick, 1990; Homewood *et al.*, 1972; Surolia and Padmanaban, 1991; Slater

N. L. Hammond · S. Choi · P. Carvalho · H. Liu · M. A. Avery (✉)
Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, MS 38677-1848, USA
e-mail: mavery@olemiss.edu

S. Khan · M. A. Avery
National Center for Natural Products Research, University of Mississippi, University, MS 38677-1848, USA

M. A. Avery
Department of Chemistry and Biochemistry, University of Mississippi, University, MS 38677-1848, USA

and Cerami, 1992). The most prevalent theory is the interference in heme polymerization, an essential detoxification mechanism of the free heme from red blood cell hemoglobin. The mechanism of action of chloroquine involves entry into the food vacuole, possibly by diffusion of the free base across membranes (Vangapandu *et al.*, 2007; Egan and Marques, 1999). After accumulation of the drug in the food vacuole, possibly by pH trapping of the protonated drug, chloroquine forms π–π complexes with heme ferriprotoporphyrin IX (Fe(III)PPIX) (Egan *et al.*, 1996; Shelnutt, 1983). Chloroquine inhibits the formation of hemozoin by forming this complex and exerts its toxic effect in the form of Fe(III)PPIX-chloroquine (Bray *et al.*, 1999).

Figure 1 shows several published anti-malarial compounds. Metaquine **4** has been found to have potent in vitro anti-malarial activity against resistant strains of Plasmodium with an IC_{50} of 0.17 μM (Dascombe *et al.*, 2005). The position of the diamine in metaquine has previously been shown to have decreased activity if ortho or para. The recently published compound **5** has been reported to have an IC_{50} of 0.6 and 0.8 nM against the D6 and W2 clones, respectively. The structural similarity between **4** and **5** may correspond to the activity we have observed with compound **6**. The site of action of **5** has yet to be determined but seems to be effective against resistant strains of the malaria parasite. We report herein the synthesis and biological activity of a novel anti-malarial agent **6** found through the screening of a compound library.

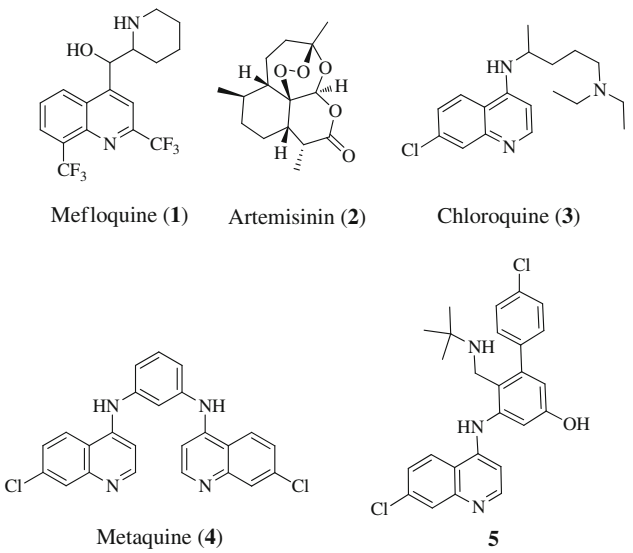


Fig. 1 Known antimalarial compounds based on quinoline scaffold and artemisinin

Chemistry

Retro synthetic analysis envisions a convergent approach to the synthesis of compound **6**. After synthesis of fragments **7** and **8**, a simple coupling reaction would lead to target compound **6**. Major considerations when evaluating the synthetic viability of fragment **7** are the one carbon linker between the two phenyl moieties. Though one carbon linkers may be found in the literature, the presence of the diamine functionality directly on the aromatic ring limits synthetic choices as it may interfere in the coupling process. Though an obvious choice is to proceed with 3,5-dinitrobenzene, the meta position of this compound is highly deactivated. Additional consideration in the synthesis of fragment **8** is the synthetic viability of the synthesis of a one carbon linker between the imidazole and the phenyl moiety. Although substitution of the 4 position of the imidazole ring is shown in the literature, it is always difficult to substitute one carbon removed from a phenyl moiety. Also taken into consideration was the tin free synthesis of this fragment, as use of tin in the synthesis of pharmaceuticals is inappropriate due to toxicity concerns (Scheme 1).

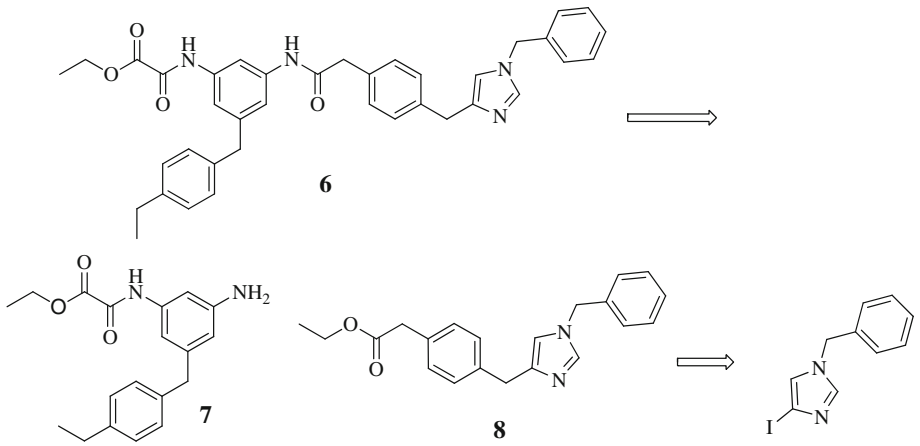
Synthesis of 7

The commercially available boronic acid **9** and benzyl chloride **10** were coupled together under previously mentioned Suzuki conditions to yield compound **11**. This step is of interest because it involves the coupling of a σ bond and π bond system, which is uncommon. The dinitro **11** was then reduced using zinc dust to give the corresponding diamine derivative **12**. One of the free amines from compound **12** was treated with ethyl oxoacetate to yield the required monoamide **7**. Amide formation was consistently a low yielding reaction (~35%). However, changing the temperature, concentration, and stoichiometry of the ethyl oxalate did not result in increased yields of the required product **7** (Scheme 2).

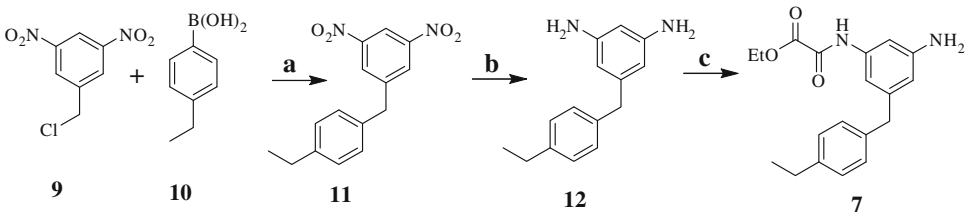
Synthesis of 8

After successful synthesis of compound **7**, we then turned our attention to the synthesis of the second fragment **8**, as seen in Scheme 3. The commercially available 4-(bromomethyl)phenylacetic acid (**13**) was esterified to yield compound **14**. The synthesis of the benzyl protected monoiodoimidazole (**15**), as previously published (Lovely *et al.*, 2007), begins with the diiodination of commercially available imidazole in the presence of potassium iodide and iodine under basic conditions. Subsequent neutralization with acetic acid precipitated the diiodo-1H-imidazole product. The 4,5-diiodo-1H-imidazole was dehalogenated

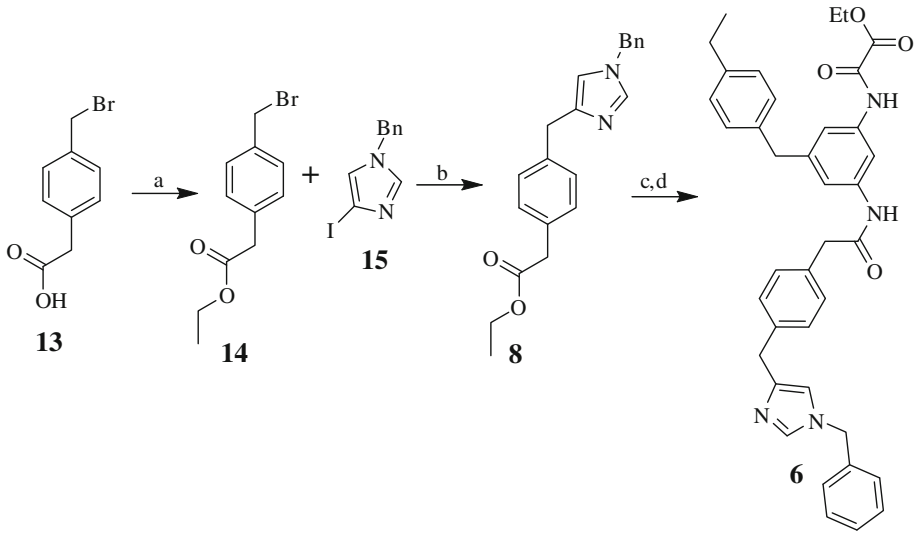
Scheme 1 Retrosynthetic analysis of the lead compound



Scheme 2 Synthesis of fragment **7**. Reagents and conditions: (*a*) DME, H_2O, Na_2CO_3, $Pd(PPh_3)_4$, reflux 24 h (96%); (*b*) AcOH, Zn dust, rt, 30 min (86%); (*c*) ethyl chlorooxoacetate, Et_3N, DCM



Scheme 3 Synthesis of fragment **8**. Reagents and conditions: (*a*) EtOH, H_2SO_4, 24 h; (*b*) i **15**, EtMgBr, DCM, 30 min. ii $ZnCl_2$, 3 h, iii **14**, $Pd(Ph_3)_4$, reflux 24 h; (*c*) THF:H_2O (1:1), LiOH, 24 h (86%); (*d*) **7**, pyridine, EDCI, DMAP, HOBt, rt 24 h (49%)



to 4-iodo-1H-imidazole by refluxing with sodium sulfite as previously published (Panosyan and Still, 2001). The monoidodide was then protected using benzyl chloride and sodium hydride in DMF to afford **13**. The benzyl protected imidazole **15** was then coupled with **14** via Grignard reagent followed by transmetallation with $ZnCl_2$ under Negishi coupling conditions to yield fragment **8**. Compound **8** was subsequently converted to the acid using LiOH. The acid was dissolved in pyridine and coupled with the monoamide derivative **7** under classical peptide coupling conditions. The final target compound **6** was evaluated for biological activity against malaria.

Results and discussion

In vitro assay

The target amide **6** and synthetic intermediates were submitted for biological evaluation. Compounds **6** and **8**

showed activity against the *Plasmodium* D6 and W2 clones, and compounds **6** and **12** showed activity against *Leishmania donovani*. Additionally, **6** and synthetic intermediates were submitted for evaluation against *Candida albicans*, *Escherichia coli*, *Cryptococcus neoformans*, methicillin-resistant *Staphylococcus aureus* (MRS), *Mycobacterium intracellulare*, and *Aspergillus fumigatus* but showed no activity. Though the activity against *Leishmania* is negligible, the activity against the plasmodial clones appears significant due to little reduction in activity against the chloroquine resistant W2 clone. Compound **6** shows structure similarity to the previously published metaquine (**4**) with substituted meta benzyl amines. However, **6** lacks the 7-chloroquinoline moiety deemed to be necessary for the activity seen with metaquine. This would suggest that the chloroquinoline moiety may not be entirely responsible for the observed activity. In addition, the previously published compound **5** shows remarkable activity against chloroquine resistant strains of the parasite (Table 1).

Conclusions

The emergence and spread of new chloroquine resistant strains of P*lasmodium faliparum* mandate the identification of novel pathways to combat the malaria parasite. Herein we have reported the identification of a novel inhibitor of *Plasmodium falciparum* that has a negligible decrease in activity against chloroquine resistant strains of the parasite. This, along with other published reports, may indicate a novel target for the treatment of chloroquine resistant malaria. The authors have under taken a structure activity relationship study to further investigate the potential of this novel lead in the treatment of *Plasmodium falciparum*.

Table 1 In vitro assay evaluation

Compound	*Plasmodium falciparum*		*Leishmania donovani*	
	D6 clone IC_{50} (μM)	W2 clone IC_{50} (μM)	IC_{50} (μM)	IC_{90} (μM)
6	6.5	5.5	41	–
7	–	–	–	–
8	8.0	6.3	–	–
11	–	–	–	–
12	–	–	69	134
14	–	–	–	–
15	–	–	–	–
Amphotericin B	–	–	1.2	2.5
Chloroquine	0.4	0.05	–	–

Experimental

General

General methods

All chemicals were purchased from Aldrich and Lanchester. All reactions were performed under an argon atmosphere in flame dried glassware. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker APX400 at 400 and 100 MHz, respectively. The high resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-Tof Micro mass spectrometer with a lock spray source. Thin-layer chromatography (TLC) was used to monitor reaction progress using pre-coated silica gel G or GP Uniplates from Analtech. Flash Chromatography was performed on silica gel 60 (Scientific Adsorbents Incorporated (SAI)). Chemical names were generated using ACD Labs 6.0 software.

Chemistry

*Ethyl 2-(3-(2-(4-((1-benzyl-1H-imidazol-4-yl) methyl)phenyl)acetamido)-5-(4-ethylbenzyl) phenylamino)-2-oxoacetate (**6**)*

Ethyl 2-(4-((1-benzyl-1H-imidazol-4-yl)methyl)phenyl)acetate (**8**) was dissolved in THF:H_2O (1:1) and stirred with LiOH for 24 h. The THF was evaporated and 1N HCl was added dropwise to the stirred solution. The white precipitate was filtered off and dried to yield the corresponding acid (80 mg, 86% yield). 2-(4-((1-benzyl-1H-imidazol-4-yl)methyl)phenyl)acetic acid (85 mg, 0.276 mmol) was dissolved in dry pyridine (10 ml). Following the addition of EDCI (105 mg, 0.552 mmol), DMAP (7 mg, 0.0552 mmol), and HOBt (75 mg, 0.552 mmol) the reaction was allowed to stir at room temperature under argon for 1 h at which point the amine (**7**) was added and stirred for 20 h. Pyridine was evaporated and the residue was purified by column chromatography on silica gel, eluting with Chloroform/MeOH (95:5) to afford 83 mg (49% yield) of **6**. ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.18 (t, 3H, $J = 7.5$); 1.34 (t, 3H, $J = 7.0$); 2.56 (q, 2H, $J = 7.4$); 3.54 (s, 2H); 3.81 (s, 2H); 3.85 (s, 2H); 4.32 (q, 2H, $J = 7.0$); 4.98 (s, 2H); 6.56 (s, 1H); 7.05 (m, 2H); 7.13 (m, 4H); 7.17 (m, 2H); 7.24 (m, 3H); 7.31 (m, 2H); 7.32 (m, 2H); 7.43 (s, 1H); 7.62 (s, 1H); 8.28 (s, 1H); 8.97 (s, 1H). ^{13}CNMR ($CDCl_3$, 100 MHz): δ 14.0, 15.6, 28.4, 34.5, 41.5, 44.1, 50.8, 63.6, 109.3, 116.1, 116.5, 117.5, 127.4, 128.0, 128.2, 128.8, 129.0, 129.4, 132.2, 136.2, 136.8, 137.0, 137.6, 138.9, 139.3, 142.1, 142.2, 143.4, 154.0, 160.7, 169.8. LCMS: calc for $C_{38}H_{38}N_4O_4Na$ *m/z* 638.28, found 637.94 $[M\text{-}H]^-$. IR 2963, 1685, 1605, 1552, 1454, 1297, 1202, 748 cm^{-1}.

*Ethyl 2-(3-amino-5-(4-ethylbenzyl)phenylamino)-2-oxoacetate (**7**)*

The amine **12** (425 mg, 1.88 mmol) was dissolved in dry dichloromethane with $(iPr)_2EtN$ (0.327 ml, 1.88 mmol) and cooled to 0°C while stirring. Ethyl 2-chloro-2-oxoacetate (0.104 ml, 0.94 mmol) was slowly added to the reaction and allowed to stir for 2 h. The reaction mixture was washed with water, then brine and dried over magnesium sulfate and concentrated. The residue was purified by column chromatography on silica gel, eluting with acetone/hexanes (5:95) to afford 104 mg (34% yield) of **7**. ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.25 (t, 3H, $J = 8$); 1.42 (t, 3H, $J = 8$); 2.64 (q, 2H, $J = 8$); 3.76 (s, 2H); 3.84 (s, 2H); 4.39 (q, 2H, $J = 8$); 6.34 (s, 1H); 6.65 (s, 1H); 7.13 (m, 5H); 8.80 (s, 1H). ^{13}CNMR ($CDCl_3$, 100 MHz): δ 14.0, 15.6, 28.4, 41.4, 63.6, 104.3, 110.5, 112.8, 128.0, 128.9, 137.4, 137.8, 142.1, 143.6, 147.5, 153.8, 161.1. LCMS: calc for $C_{19}H_{22}N_2O_3$ *m/z* 327.17, found 327.17 $[M\text{-}H]^-$. IR 3327, 1687, 1608, 1562, 1279, 1222, 1021, 820 cm^{-1}.

*Ethyl 2-(4-((1-benzyl-1H-imidazol-4-yl)methyl)phenyl)acetate (**8**)*

1-benzyl-4-iodo-1H-imidazole was dissolved in dry THF at 0°C and 1 M EtMgBr in THF (0.78 ml, 0.78 mmol) was added dropwise to the stirred solution and allowed to stir for 1 h. Anhydrous $ZnCl_2$ (212 mg, 1.56 mmol) was added to the reaction and allowed to stir. After 2 h, the bromide **13** and the catalyst Pd(PPh3)4 were added and the reaction was refluxed for 24 h. After 24 h, the reaction was quenched with saturated ammonium chloride (5 ml) and the resulting mixture was extracted from H_2O (3 × 20 ml) with chloroform. The organic layers were combined and dried over $MgSO_4$. The residue was purified by column chromatography on silica gel, eluting with chloroform/MeOH (95:5) to afford 105 mg (31% yield) of **8**. ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.10 (t, 3H, $J = 8$); 3.43 (s, 2H); 3.78 (s, 2H); 3.99 (q, 2H, $J = 8$); 4.80 (s, 2H); 6.42 (s, 1H); 7.12 (m, 4H); 7.16 (m, 2H); 7.31 (m, 2H); 7.55 (m, 1H); 7.60 (m, 1H). ^{13}CNMR ($CDCl_3$, 100 MHz): δ 14.1, 34.6, 40.9, 50.5, 60.6, 116.2, 127.2, 128.0, 128.4, 128.5, 128.8, 129.0 (2C), 129.2 (2C), 131.9, 132.0, 133.0, 136.4, 136.9, 139.1, 142.4, 171.5. LCMS: calc for $C_{21}H_{22}N_2O_2$ *m/z* 335.17, found 335.17 $[M\text{-}H]^-$. IR 2920, 1729, 1498, 1367, 1251, 1153, 1030, 721 cm^{-1}.

*1-(4-Ethylbenzyl)-3,5-dinitrobenzene (**11**)*

1-(chloromethyl)-3,5-dinitrobenzene (10.05 g, 46.4 mmol), 4-ethylphenylboronic acid (13.0 g, 92.8 mmol), sodium carbonate (9.84 g, 92.9 mmol), Tetrakis(triphenylphosphine)palladium(0) (250 mg, 0.216 mmol), and dichlorobis (triphenylphospine) palladium (II) (250 mg, 0.356 mmol) were dissolved in 1,2-dimethoxyethane (150 ml) and water (50 ml) and refluxed for 24 h. The reaction mixture was allowed to cool to room temperature and extracted with DCM (2 × 50 ml). The combine organic layers were then washed with water, brine, and dried over magnesium sulfate. The organic layer was concentrated and the residue was chromatographed on silica gel using ethyl acetate: hexane (10:90) to afford 12.738 g (96% yield) of **9**. ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.27 (t, 3H, $J = 7.6$); 2.67 (q, 2H, $J = 7.6$); 4.20 (s, 2H); 7.15 (d, 2H, $J = 8$); 7.22 (d, 2H, $J = 8$); 8.40 (s, 2H); 8.90 (s, 1H). ^{13}CNMR ($CDCl_3$, 100 MHz): δ 15.4, 28.4, 41.1, 116.8, 128.7 (2C), 128.8 (2C), 128.9 (2C), 134.9, 143.9, 146.1, 148.6. LCMS (APCI): calc for $C_{15}H_{14}N_2O_4$ *m/z* 287.10, found 287.23 $[M\text{-}H]^-$. IR: 3105, 2971, 2929, 1530, 1513, 1339, 1078, 725 cm^{-1}.

*5-(4-Ethylbenzyl)benzene-1,3-diamine (**12**)*

1-(4-ethylbenzyl)-3,5-dinitrobenzene (182 mg, 0.63 mmol) was dissolved in acetic acid (20 ml) with zinc dust (2.2 g, 38 mmol) at 0°C and stirred for 3 h. The reaction was filtered through Celite and washed thoroughly with ethyl acetate and concentrated. The organic layer was washed with water, $NaHCO_3$, and brine. The resulting residue was chromatographed on silica gel using ethyl acetate: hexanes (40:60) to afford 109 mg (86% yield) of **10**. ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.37 (m, 3H) 2.75 (m, 2H); 3.55 (s, 4H); 3.77 (s, 2H); 5.86 (s, 1H); 6.02 (s, 2H); 7.24 (s, 4H). ^{13}CNMR ($CDCl_3$, 100 MHz): δ 15.9, 28.6, 41.7, 100.2, 106.9 (2C), 128.0 (2C), 129.1 (2C), 138.7, 141.9, 143.7, 147.8. LCMS: calc for $C_{15}H_{18}N_2$ *m/z* 227.15, found 227.15 $[M\text{-}H]^-$. IR: 3417, 3326, 2967, 1598, 1512, 1354, 1187, 818 cm^{-1}.

*1-benzyl-4-iodo-1H-imidazole (**13**)*

Imidazole (7.06 g, 103.7 mmol) was dissolved in a solution of sodium hydroxide (12 g, 213.8 mmol) in water (300 ml). A solution of iodine dissolved in 600 ml of 20% sodium iodide was added dropwise to the stirred solution of imidazole and allowed to stir for 8 h. The resulting mixture was neutralized with concentrated acetic acid to give a white precipitate. The precipitate was filtered, washed with water, dissolved in ethanol and evaporated to dryness to afford 22.7 g (69% yield) diiodoimidazole. Diiodoimidazole (22.7 g, 71 mmol) was dissolved in 30% ethanol with sodium sulfite (134 g, 1065 mmol) and refluxed at 98°C for 24 h. The reaction was allowed to cool and extracted three times with ethyl ether and concentrated to afford 9.272 g (67% yield) of 4-iodo-1H-imidazole **11**. ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.20 (s, 1H); 7.66 (s, 1H). LCMS: calc for $C_3H_3IN_2$ *m/z* 194.94, found 195.09 $[M\text{-}H]^-$.

IR 2787, 2590, 1820, 1433, 1163, 952, 828, 758 cm^{-1}. Sodium hydride (60% in mineral oil, 227 mg, 5.9 mmol) was dissolved in dry DMF under argon at 0°C to which 4-iodo-1H-imidazole (1.041 g, 5.37 mmol) was added and stirred for 30 min. To this mixture was added benzyl bromide (0.7 ml, 5.9 mmol) dropwise at 0°C and then allowed to slowly warm to room temperature and stir for 16 h. The reaction was quenched with water and extracted with EtOAc from water followed by saturated sodium chloride and dried over $MgSO_4$. The residue was purified by column chromatography on silica gel, eluting with Hexanes/EtOAc (70:30) to afford 707 mg (50% yield) of **12**. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.01 (s, 1H); 6.91 (s, 1H); 7.11 (d, 2H, $J = 8$); 7.29 (m, 3H); 7.37 (s, 1H). ^{13}CNMR ($CDCl_3$, 100 MHz): δ 51.1, 124.9, 127.6, 128.6, 129.1, 135.4, 139.0. LCMS: calc for $C_{10}H_9IN_2$ *m/z* 284.98, found 285.20 $[M-H]^-$. IR 3104, 1508, 1444, 1229, 1214, 1197, 934, 715 cm^{-1}.

*Ethyl 2-(4-(bromomethyl)phenyl)acetate (**14**)*

2-(4-(bromomethyl)phenyl)acetic acid (2 g, 12 mmol) and H_2SO_4 (2 ml) were dissolved in EtOH (30 ml). After stirring for 24 h, the EtOH was removed under vacuum and the resulting mixture was extracted from H_2O (3 × 20 ml) with EtOAc. The organic layers were combined and dried over $MgSO_4$. The residue was purified by column chromatography on silica gel, eluting with Hexanes/EtOAc (85:15) to afford 2.2 g (86% yield) of Ethyl 2-(4-(bromomethyl)phenyl)acetate **13**. ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.28 (t, 3H, $J = 7.1$); 3.63 (s, 2H); 4.18 (q, 2H, $J = 7.1$); 4.50 (s, 2H); 7.29 (d, 2H, $J = 8.1$); 7.37 (d, 2H, $J = 8.1$). ^{13}CNMR ($CDCl_3$, 100 MHz): δ 14.2, 33.2, 41.1, 60.9, 129.3 (2C), 129.7 (2C), 134.5, 136.6, 171.2. IR 2977, 1721, 1368, 1338, 1219, 1158, 1020, 769 cm^{-1}.

In vitro assays

Compounds were screened for antileishmanial (Rao *et al.*, 2004), antimalarial (El Sayed *et al.*, 1996; Peng *et al.*, 2010), and anti-microbial activity (Peng *et al.*, 2010) by the National Center for Natural Products Research (NCNPR), and assays were performed according to previously published procedures. Briefly, in vitro activity against chloroquine sensitive (D6, Sierra Leone) and chloroquine-resistant (W2, Indo China) strains of *P. falciparum* was measured by determining the plasmodial LDH activity. The compounds to be tested were first dissolved in DMSO (2 mg/ml). As previously described, the samples were serial diluted in 96-well plates by addition to a 200 µl suspension of *P. falciparum* culture and incubated at 37°C in 5% CO_2 for 72 h. The plasmodial LDH activity was determined using the Malstat reagent, and chloroquine was used as positive control (Peng *et al.*, 2010).

In vitro antimicrobial activity was determined as previously described (Peng *et al.*, 2010). All test organisms were supplied by American Type Culture Collection (ATCC). Each compound was serial diluted in 20% DMSO/saline and added in duplicate to 96-well plates of microbe suspensions. Positive controls of ciprofloxacin and amphotericin B were used in each assay. All organisms were evaluated using an EL-340 Biokinetics Reader at 630 nm before and after incubation. In vitro antileishmanial activity was determined as previously described (Avery *et al.*, 2003). Briefly, compounds were tested on a transgenic cell line of *Leishmania donovania* that expressed firefly luciferase. The samples were serial diluted in 96-well plates by addition to a suspension of leishmania promastigote culture (2×10^6 cells/ml) and incubated at 26°C in 5% CO_2 for 72 h. The growth of the leishmania promastigotes was determined by luciferase assay using Steady Glo (Promega), and amphotericin B was used as positive control. All compounds were tested at six different concentrations in duplicate, and IC_{50} values were determined using the mean values to generate growth inhibition curves (Avery *et al.*, 2003).

Acknowledgments We thank the CDC cooperative agreements 1UO1 CI000211-03 and 1UO1 CI000362-01 for financial support. Additionally, investigation was conducted in a facility constructed with support from Research Facilities Improvement Program Grant Number C06 Rr-14503-01 from the National Center for Research Resources, National Institutes of Health.

References

Avery MA, Muraleedharan KM, Desai PV, Bandyopadhyaya AK, Furtado MM, Tekwani BL (2003) Structure-activity relationships of the antimalarial agent artemisinin. 8. Design, synthesis, and CoMFA studies toward the development of artemisinin-based drugs against leishmaniasis and malaria. J Med Chem 46:4244–4258

Bray PG, Janneh O, Raynes KJ, Mungthin M, Ginsburg H, Ward SA (1999) Cellular uptake of chloroquine is dependent on binding to ferriprotoporphyrin IX and is independent of NHE activity in *Plasmodium falciparum*. J Cell Biol 145:363–376

Butcher GA, Sinden RE, Curtis C (2000) Malaria: new ideas, old problems, new technologies. Parasitol Today 16:43–44

Dascombe MJ, Drew MGB, Morris H, Wilairat P, Auparakkitanon S, Moule WA, Alizadeh-Shekalgourabi S, Evans PG, Lloyd M, Dyas AM, Carr P, Ismail FMD (2005) Mapping antimalarial pharmacophores as a useful tool for the rapid discovery of drugs effective in vivo: design, construction, characterization, and pharmacology of metaquine. J Med Chem 48:5423–5436

Egan TJ, Marques HM (1999) The role of heme in the activity of chloroquine and related antimalarial drugs. Coord Chem Rev 190–192:493–517

Egan TJ, Ross DC, Adams PA (1996) The mechanism of action of quinolines and related anti-malarial drugs. S Afr J Sci 92:11–14

El Sayed KA, Dunbar DC, Goins DK, Cordova CR, Perry TL, Wesson KJ, Sanders SC, Janus SA, Hamann MT (1996) The marine environment: a resource for prototype antimalarial agents. J Nat Toxins 5:261–285

Homewood CA, Warhurst DC, Peters W, Baggaley VC (1972) Lysosomes, pH and the anti-malarial action of chloroquine. Nature 235:50–52

Lovely CJ, Du H, Sivappa R, Bhandari MR, He Y, Dias HVR (2007) Preparation and Diels-Alder Chemistry of 4-Vinylimidazoles. J Org Chem 72:3741–3749

Meshnick SR (1990) Chloroquine as intercalator: a hypothesis revived. Parasitol Today 6(3):77–79. Chem. Abstract Number: 112:210412

Panosyan FB, Still IWJ (2001) An efficient route to 5-iodo-1-methylimidazole: synthesis of xestomanzamine A. Can J Chem 79:1110–1114

Peng J, Kudrimoti S, Prasanna S, Odde S, Doerksen RJ, Pennaka HK, Choo YM, Rao KV, Tekwani BL, Madgula V, Khan SI, Wang B, Mayer AM, Jacob MR, Tu LC, Gertsch J, Hamann MT (2010) Structure-activity relationship and mechanism of action studies of manzamine analogues for the control of neuroinflammation and cerebral infections. J Med Chem 53:61–76

Rao KV, Kasanah N, Wahyuono S, Tekwani BL, Schinazi RF, Hamann MT (2004) Three new manzamine alkaloids from a common Indonesian sponge and their activity against infectious and tropical parasitic diseases. J Nat Prod 67:1314–1318

Ridley RG (2002) Medical need, scientific opportunity and the drive for antimalarial drugs. Nature 415:686–693

Shelnutt JA (1983) Metal effects on metalloporphyrins and on their p–p charge-transfer complexes with aromatic acceptors: urohemin complexes. Inorg Chem 22:2535–2544

Slater AF, Cerami A (1992) Inhibition by chloroquine of a novel haem polymerase enzyme activity in malaria trophozoites. Nature 355:167–169

Surolia N, Padmanaban G (1991) Chloroquine inhibits heme-dependent protein synthesis in *Plasmodium falciparum*. Proc Natl Acad Sci USA 88:4786–4790

Vangapandu S, Jain M, Kaur K, Patil P, Patel SR, Jain R (2007) Recent advances in antimalarial drug development. Med Res Rev 27:65–107