Malaria Causal Prophylactic Activity of Imidazolidinedione Derivatives

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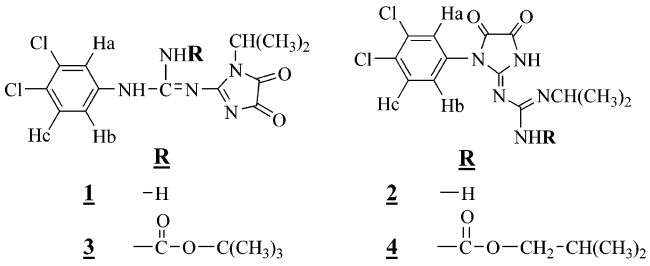
A series of acid-stable carboxamide derivatives of 2-guanidinoimidazolidinedione (**5a**−**c** and **6a**−**c**) were prepared as potential malaria prophylactic and radical cure agents. The new compounds showed moderate to good causal prophylactic activity in mice infected with *Plasmodium yoelii* sporozoites. Three compounds were further tested for causal prophylactic activity in Rhesus monkeys infected with *Plasmodium cynomolgi* sporozoites, and all showed a delay in patency from 13 to 40 days at 30 mg/kg/day × 3 days by IM dosing. Two out of four compounds tested for radical curative activity in Rhesus showed cure at 30 mg/kg/day × 3 days. The other two compounds showed delay in relapse from 16 to 68 days. Conversion of new carboxamides (**5** and **6**) to *s*-triazine derivatives (**7**) was demonstrated in mouse and human microsomal preparations and in rat plasma. The results suggest the metabolites, *s*-triazine derivatives **7**, may be the active species of the new carboxamides **5a**−**c** and **6a**−**c** prepared in this study.

Introduction

The current global situation with respect to malaria indicates that about two billion people are exposed to the disease. Each year, between 100 and 200 million new cases of infection are reported, and approximately 1 to 2 million people die due to malaria.[1−4] The situation is rapidly worsening, mainly due to unavailability of effective drugs and development of drug resistance to the existing first line drugs, such as chloroquine and pyrimethamine.[5−10] In addition to the drug resistance of the first line antimalarials drugs, the usefulness of many newer antimalarial drugs is impaired by their side effects. Multiple drug resistance in *Plasmodium falciparum* malaria continues to pose special problems for targeting the blood stages of malaria. Our product development teams for malaria prophylaxis are placing emphasis on developing new chemical entities with true causal prophylactic or radical curative properties, stopping malaria before blood stages emerge and cause clinical disease. With the exception of quinoline esters,[11] only the 8-aminoquinoline drugs such as primaquine or tafenoquine[12−14] have activity against the liver stages of *Plasmodium vivax* and *P. falciparum* malarias. However, the 8-aminoquinoline drugs cause serious lethal hemolytic side effects in glucose-6-phosphate dehydrogenase (G6PD)-deficient patients.[8] Therefore, there is an eminent need for new and safe antimalarial drugs to combat the parasites and protect the tourists traveling in the endemic areas of the world.

Recently, a series of new 2-guanidinoimidazolidinedione (**IZ**) derivatives (**1** and **2**, Scheme 1) was demonstrated to possess causal prophylactic antimalarial activity in Rhesus monkeys infected with *P. cynomolgi* sporozoites.[15] **IZ** derivatives **1** and **2** are sparingly soluble in water or common organic solvents, and the prophylactic activity is demonstrated only when the compounds are administered by subcutaneous (SC[a]) or intramuscular (IM) injection, but not orally. To improve the solubility

Scheme 1



in organic solvents, thus facilitating the purification and improving the oral absorption, a series of carbamate derivatives of **IZ**, such as **3** and **4**, was prepared.[16−18] Carbamates **3**, the most active compounds of this class, protect monkeys infected with *P. cynomolgi* sporozoites at a dose of 10 mg/kg × 3 days by IM dosing. Nevertheless, the **IZ** derivatives **1**−**4** showed very weak or no in vitro cell growth inhibition against blood stage malaria *P. falciparum* and were inactive in the Thompson mouse test against *P. berghei*, a blood stage rodent malaria.[16−18] To the best of our knowledge, this is the first class of antimalarial agents possessing activity against the liver stage malaria exclusively. From the drug resistant development point of view, drugs used exclusively for prophylaxis have much less chance of exposure to parasites than those used for treatment and, thus, have fewer problems of developing drug resistance. However, compound **3** showed poor activity by oral administration and delayed the patency (first day the parasite can be detected in blood smears after infection) of monkeys treated for only 3 days versus the untreated control. Oral efficacy is an essential consideration in the search for prophylactic drugs. The lack of oral activity of carbamates **3** and **4** in Rhesus testing may be attributed to the hydrolysis of the carbamate group in stomach acid, leading to generation of insoluble parent compounds **1** and **2**.

To overcome the acid instability of carbamates, a series of acid-stable carboxamide derivatives of **1** and **2**, such as **5a**−**c**

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[a] Abbreviations: Patency: first day the parasite can be detected in blood smears after infection; CQ: chloroquine; PO: oral administration; SC: subcutaneous injection; IM: intramuscular injection.

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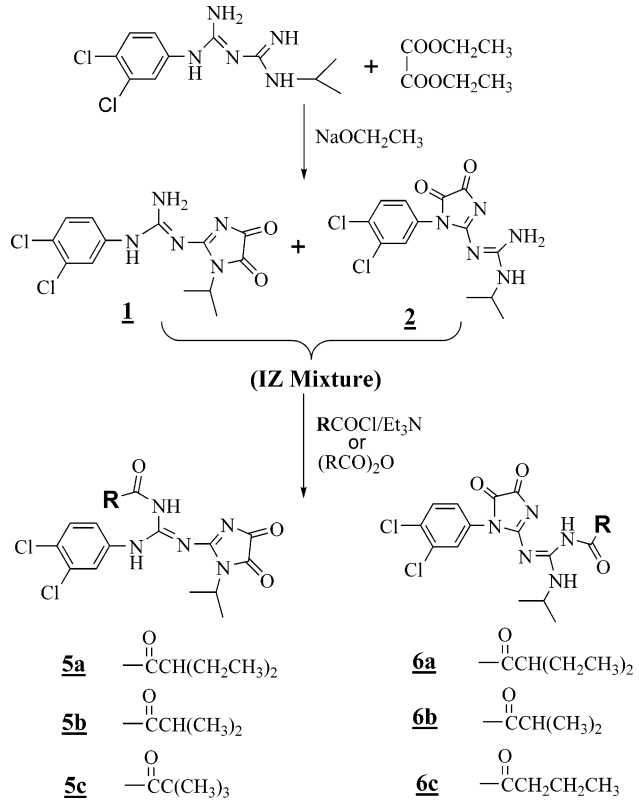
Scheme 2



and **6a**−**c**, was prepared in this study. The new compounds were tested against exoerythrocytic *P. yoelii* in mice and *P. cynomolgi* in Rhesus monkeys. In vitro metabolic stability of the new compounds in human and mouse microsomal preparations were performed.

Chemistry

A series of carboxamide derivatives **5a**−**c** and **6a**−**c** were prepared as acid-stable analogues of **3** and **4**, respectively (Scheme 2). Carboxamide derivatives **5a**−**c** and **6a**−**c** were prepared according to the same method as that used for the preparation of carbamate derivatives **3** and **4**,[16−18] using acyl chloride or anhydride instead of alkyl chloroformates as reagents. **IZ mixture** (mixture of compounds **1** and **2**, WR182393**)**[16] was obtained by treatment of chloroproguanil with diethyloxalate using $NaOCH_2CH_3$ as the catalyst. Compounds **5a** and **6a** were obtained by treatment of **IZ mixture** with 1.1 molar equiv of ethylbutyryl chloride under the catalysis of triethylamine and 4-*N*,*N*-dimethylaminopyridine (DMAP). The crude product was purified using a silica gel column to give a mixture of **5a** and **6a**. Due to the close structural similarity of **5a** and **6a**, their R_f values in various solvent systems are almost identical. Thus, separation of **5a** and **6a** by column chromatography was an impractical option. NMR spectra indicated that the product ratio of **5a** and **6a** is 1 to 2. Since compound **6a** is more soluble than **5a** in organic solvents, the separation was achieved by washing the mixture with an EtOAc/$CHCl_3$ (2:1 v/v) mixed solvent.

The same procedure was employed to prepare isobutyryl carboxamide **5b** and **6b** using **IZ mixture** as the starting material. However, only **6b** was obtained as a major product, and **5b** had to be prepared from pure compound **1**, the synthesis of which was described in our previous publication.[17,18] Anhydrides instead of acyl chlorides were used in the preparation of **5c** and **6c** because a spectrum of unknown byproducts was produced when highly reactive acyl chlorides were used. As the solubility of the **IZ Mixture** is sparingly soluble in chloroform, 5% dimethylformamide (DMF)/chloroform was used as the solvent to facilitate the reaction. Again, only **6c** was isolated when **IZ mixture** was treated with butyric anhydride. Compound **5c** was prepared using pure **1** and trimethylacetic anhydride as the starting materials.

Since **5a**−**c** and **6a**−**c** are derivatives of **1** and **2**, the structural characterization of **5a**−**c** and **6a**−**c** was based on differences in NMR spectra between compounds **1** and **2**. The chemical shifts of the methylene proton (−N−C**H**−) of the isopropyl group in **5a**−**c** and **6a**−**c** are distinctively different, with the former being more downfield shifted than the latter by ∼0.3 ppm.[16] Furthermore, the Ha proton signal of compounds **5a**−**c** (Scheme 1) also resonances ∼0.30 ppm downfield to that of the corresponding proton of **6a**−**c**. Detailed chemical shift differences between derivatives of compound **1** and **2** were discussed in the earlier publications of this series.[16,17]

Experimental Section

Melting points were determined on a Mettler FP62 melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed using HPTLC-HLF normal phase 150 micron silica gel plates (Analtech, Newark, DE). Visualization of the developed chromatogram was performed by UV absorbance or by staining with iodine vapor. Liquid chromatography was performed using a Horizon HPFC System (Biotage, Charlottesville, VA) with Flash 25M or 40M cartridges (KP-Sil Silica, 32−63 μm, 60 Å). Preparative TLC was performed using silica gel GF tapered uniplates (Analtech, Newark, DE). ^{1}H NMR and ^{13}C NMR spectra were recorded in deuteriochloroform, unless otherwise noted, on a Bruker Avance 300 and Bruker Avance 600 spectrometer (Bruker Instruments, Inc, Wilmington, DE). Chemical shifts are reported in parts per million on the δ scale from an internal standard of tetramethylsilane. Combustion analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Where analyses are indicated by symbols of the elements, the analytical results obtained were within ±0.4% of the theoretical values.

Preparation of Compound 5a. To a suspension of compound **1** (5 g, 14.6 mmol) in 100 mL of $CHCl_3$ was added, with stirring, Et_3N (4.1 mL, 2 equiv) and DMAP (179 mg, 0.1 equiv). The reaction mixture was cooled to 0 °C with an ice bath, and 2-ethylbutyryl chloride (3 mL, 1.5 equiv) was added dropwise. The reaction mixture was stirred at room temperature overnight and washed with water, and the chloroform layer was dried over Na_2SO_4 and evaporated to dryness under the reduced pressure. The residue was applied to a silica gel flash column and eluted with 2% EtOAc /$CHCl_3$ to give a white solid, which was recrystallized from hexane/$CHCl_3$ to get the desired compound as a light-yellow solid (2.6 g, 40%); mp 206.5−208.4 °C. MS, NMR, and elemental analysis data are available in the Supporting Information, S-1.

Compound **5a** was also prepared using **IZ Mixture** as the starting material, with a low yield **(**15%); mp 206−208 °C. The NMR and MS spectra of the product are identical to those of **5a** prepared from pure compound **1**.

Compound **5b** was prepared according to the same procedure as that for the preparation of compound **5a**, using pure compound **1** and isobutyryl chloride as staring materials to give a 26% yield of pale-yellow crystals; mp 188−190 °C. MS, NMR, and elemental analysis data of **5b** are available in the Supporting Information, S-1.

Compound **5c** was prepared using trimethylacetic anhydride as the acylating agent to yield 23% of the desired product after purification; mp 183.3−190.7 °C. MS, NMR, and elemental analysis data are available in the Supporting Information, S-1.

Preparation of Compound 6a. To the suspension of **IZ mixture** (2.0 g, 5.85 mmol) in 20 mL of dry chloroform were added triethylamine (2.5 mL), DMAP (50 mg, 0.4 mmol), and 2-ethylbutyryl chloride (2.52 mL, 18.3 mmol), successively under the ice

Table 1. Causal Prophylactic Activity in *P. Yoelii* Sporozoite-Challenged Mouse and Metabolic Stability in Human and Mouse Microsomal Preparations

compound #	dose (mg/kg)/ day	# days treated	total dose (mg)	route	# protected/ # mice used at day 6	metabolic stability $t_{1/2}$ (min) human	mouse
5a	10	3	30	PO	4/5	>60	>60
	5	3	15		1/5		
	2.5	3	7.5		2/5		
5b	160	3	480	PO	1/5	>60	40
	40	3	120		2/5		
	10	3	30		2/5		
5c	160	3	480	PO	0/5	>60	>60
	40	3	120		0/5		
	10	3	30		1/5		
6a	160	1	160	PO	5/5	<10	<10
	40	1	40		4/5		
	10	1	10		2/5		
6b	160	1	160	PO	5/5	<10	<10
	40	1	40		5/5		
	10	1	10		2/5		
6c	160	1	160	PO	5/5	<10	<10
	40	1	40		5/5		
	10	1	10		5/5		
	5	1	5		3/5		
	2.5	1	2.5		1/5		
7a	160	3	480	PO	2/5	>60	>60
	40	3	120		2/5		
	10	3	30		3/5		

bath temperature. The reaction mixture was gradually warmed up to room temperature. After stirring overnight, the mixture was quenched with water and extracted with $CHCl_3$ three times. The chloroform extracts were combined, washed with water three times, and dried over Na_2SO4. The solvent was filtered and evaporated to dryness under reduced pressure. The crude product was purified by flash silica gel column chromatography using 35% EtOAc/hexane as the eluent to give a white product. Recrystallization from EtOAc/$CHCl_3$ yielded pure compound **6a** as white needle crystals (700 mg, 26%); mp 160 °C. MS, NMR, and elemental analysis data of **6a** are available in the Supporting Information, S-1 and S-2.

Compound **6b** and **6c** were obtained following the same procedure as that for **6a** by treatment of **IZ mixture** with isobutyryl and butyryl anhydride, respectively. MS, NMR, and elemental analysis data of **6b** and **6c** are available in the Supporting Information, S-1 and S-2.

Antimalarial Studies. 1. *Plasmodium yoelii* Sporozoite-Induced Test in Mice (SM Test). The causal prophylactic antimalarial activity of the new derivatives was assessed in a *P. yoelli* sporozoite-challenged mouse model by Dr. Arba Ager of the University of Miami, Miami, FL. The detailed procedure of sporozoites harvest and drug tests are disclosed in the previous reports of this series.[16−18] The results are shown in Table 1.

2. *Plasmodium cynomolgi* Sporozoite-Induced Test in Rhesus Monkeys. The causal prophylactic and radical curative antimalarial activity of the new derivatives **5a** and **6a** was assessed in a *P. cynomolgi* sporozoite-challenged Rhesus monkey model. The detailed procedure of sporozoites harvest and drug tests are described in the previous reports.[16] The results are shown in Table 2.

3. Radical Cure Test in Rhesus Monkeys. Assessment of radical curative activity of the test compounds was carried out using the monkeys' developed parasitemia during the causal prophylactic experiments when the test compounds showed no or weak activity. Monkeys were treated with chloroquine (10 mg/kg/day) orally for 7 consecutive days, and the test compounds by IM for 3 consecutive days after the parasitemia level reached 5,000 parasites/mm^3. Chloroquine at 10 mg/kg/day × 7 days eliminated the blood stage parasites but not the liver stage hypnozoites. Compounds with antihypnozoite activity will delay the relapse or radically cure the infection.

To evaluate the radical curative properties, daily blood samples were followed for 21 days, 3 times per week for 4 weeks, and then 2 times weekly until 100 days after the last day of test compound administration. Parasite clearance should occur in all animals treated with chloroquine. Relapse was expected in the control group. Relapse in the treated group indicates failure of the test compounds. Monkeys that showed no relapse after 100 days were considered radically cured. Relapses of the control monkeys were treated with chloroquine once daily for 7 days, and they were observed for the second relapse. Relapse in experimental animals and the second relapse of the control monkeys were treated with the standard 7 day oral CQ and primaquine (1.78 mg base/kg). After standard treatment, blood smears were monitored daily for 4 consecutive days of negative results and 2 times weekly for 2 weeks. The results are shown in Table 3.

4. Metabolic Stability Study. The metabolic stability assay sample preparation was performed in a 96 well plate on a TECAN Genesis robotic sample processor following WRAIR SOP SP 01−02. All incubations were carried out in 0.1 M sodium phosphate buffer (pH 7.4) in the presence of a NADPH-regenerating system (NADP sodium salt, $MgCl_2 \cdot 6H_2O$, and glucose-6-phosphate). The test drug (1 μM), microsomes (0.5 mg/mL total protein), buffer, and the NADPH-regenerating system were warmed to 37 °C, and the reaction was initiated by the addition of glucose-6-phosphate dehydrogenase (G6PD). Samples were quenched at 0, 10, 30, and 60 min using an equal volume of cold methanol. Samples were centrifuged to pellet the proteins, and the supernatant was analyzed by LC-MS/MS using fast LC gradient or isocratic methods. Chromatograms were analyzed using the mass spectrometry software Xcalibur QuanBrowser. Concentrations of the parent drug remaining at each time point were calculated using the unknown peak areas and corresponding calibration curves. If sufficient sensitivity was not obtained for the test drugs at 1 μM, 10 μM drug and 1 mg/mL total microsomal protein were used. In order to calculate the half-life, a first-order rate of decay was assumed. A plot of the natural log (ln) of the drug concentration versus time was generated, where the slope of that line was $-k$. The half-life was calculated as 0.693/k.

For metabolite identification, samples were prepared as described above with human liver microsomes. Additional samples were prepared with each drug using mouse, rat, and Rhesus monkey liver microsomes. Samples were separated using a LC gradient method and analyzed by full-scan LC-MS and LC-MS/MS. Parent compounds and putative metabolites were all fragmented, and these MS/MS experiments were used in combination with the no-NADPH control experiments to confirm the assignment of peaks as metabolites. These MS/MS data were also used to do preliminary structural elucidation.

Results and Discussion

By single-dose oral treatment, all three carboxamide derivatives **6a**−**c** exhibited profound activity in the *P. yoelii* sporozoite-challenged mouse model with a minimum active dose (MAD) of <10 mg/kg. In contrast, **5a**−**c** showed much weaker causal prophylactic activity than that of **6a**−**c** in the same test, with MAD > 160 mg/kg. However, when **5a**−**c** were administered once a day for 3 consecutive days (−1, 0, +1 day), starting a day before the sporozoites challenge, the MAD of **5a**, **5b**, and **5c** improved to 7.5, <30, and >480 mg/kg, respectively (Table 1). Likewise, triazine **7a** showed weak single-dose oral activity, but substantially better efficacy was observed when the mice were treated for 3 consecutive days (Table 1).

Selected new compounds **5a**, **5c**, and **6a** were further tested for causal prophylactic (CP) and radical cure (DB) activities in Rhesus moneys infected with *P. cynomolgi* sporozoites. For a causal prophylactic test, the drug was administered by IM once a day for three consecutive days, starting a day before the parasites' inoculation. Blood smears were taken to monitor the development of parasitemia formation during the course of

Table 2. Causal Prophylactic Activity of **3**, **5a**, **5c**, and **6a** in *P. Cynomolgi* Sporozoite-Challenged Rhesus Monkeys

monkey group #[a]	compound #	dose (mg/kg)	days treated[c]	route	results	parasite patency (days post-inoculation)
1	control	N/A[b]	1 daily for 3 days	IM	valid control	8 days 8 days
2	**3**	30	1 daily for 3 days	IM	delayed patency[d] for 39−60 days	47 days 68 days
3	**5a**	30	1 daily for 3 days	IM	delayed patency for 4−9 days	17 days 13 days
4	**5c**	30	1 daily for 3 days	IM	delayed patency for 13−32 days	40 days 21 days
5	**6a**	30	1 daily for 3 days	IM	delayed patency for 5−7 days	13 days 15 days

[a] Two monkeys per dose group. [b] At the same volume as other experimental groups. Maximum DMSO volume per site is 1 mL for 2 injection sites (one in each thigh). [c] Drugs were dissolved in DMSO and given on the day before, on the day, and a day after (−1, 0, +1 day) sporozoite inoculation. [d] First day the parasite can be detected in blood smears after infection.

Table 3. Radical Curative Activity of Compounds **3**, **5a**, **5c**, and **6a** in Relapsed Rhesus Monkeys

group no.	drug 1	drug 2	dose (mg/kg)	# doses per day	route	results	days post-treatment
1	CQ, 10 mg/ kg × 7, PO	none	oral	relapse	9 days 9 days		
	5a	30	1 daily for 3 days	IMradical curative	no relapse	delayed relapse	16 days
	3	**5c**	30	1 daily for 3 days	IM	delayed relapse	38days 41 days
CQ, 10 mg/ kg × 7, PO	4	**6a**	30	1 daily for 3 days	IM	delayed relapse	26days 16 days
	5	**3**	30	1 daily for 3 days	IM	delayed relapse	53 days 68 days
	6	**3**	30	1 daily for 3 days	IM	delayed relapse radical curative	55 days no relapse

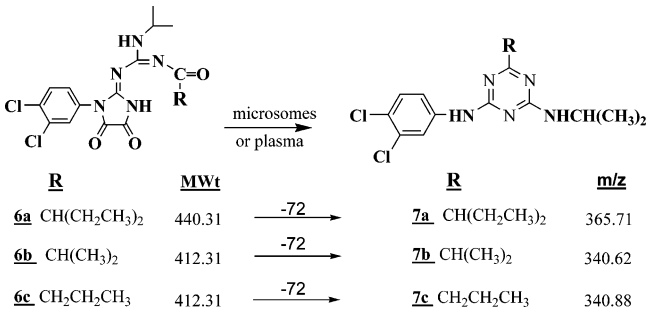
experiment, first on day 6 after parasite inoculation and continued daily for 21 days. After that, the parasite count was checked 3 times per week for 4 weeks and then 2 times weekly until 100 days after the last day of testing compound treatment. Carbamate derivative **3**, the most active compound of the series, was used as the positive control.[16]

Results (Table 2) indicated that all three new compounds delayed patency of treated monkeys from 4 days (**5a**) to more than a month (**5c**) as compared with the untreated control monkeys. Although compound **3**, the positive control, showed 100% protection (parasite free for >100 days) at 10 mg/kg/day × 3 in previous experiments,[16] the results of this experiment showed compound **3** only delayed patency of the treated monkeys for 39 days for one monkey and 60 days for other, as compared with untreated monkeys. The discrepancy in prophylactic efficacy from one experiment to the other may be the result of differences in viability of the sporozoites used. Compounds **5a** and **6a** are 2-ethybutyrylamide derivatives of **1** and **2**, respectively, and are about equal in efficacy, with delayed patency for 4−9 days. Compound **5c**, a carboxamide analogue of **3**, showed superior protective activity to that of **5a** and **6a**, with delayed patency 13 days for one of the two treated monkeys and 32 days for another.

The data from the liver stage malaria mouse model used in this study appear to provide poor prediction of the monkey test results. Compound **6a** showed superior efficacy to **5a** and **5c** in the mouse test, yet the latter compounds (**5a** and **5c)** showed equal or better causal prophylactic activity than that of the former compound **6a**. Especially, compound **5c**, which showed no activity in the mouse test either by single or 3 day treatment, exhibited superior activity in Rhesus monkeys to that of **6a**.

Monkeys which developed parasitemia in the causal prophylactic test were used for the radical curative test. Test compounds were co-administered with chloroquine for 7 days to eliminate

Scheme 3

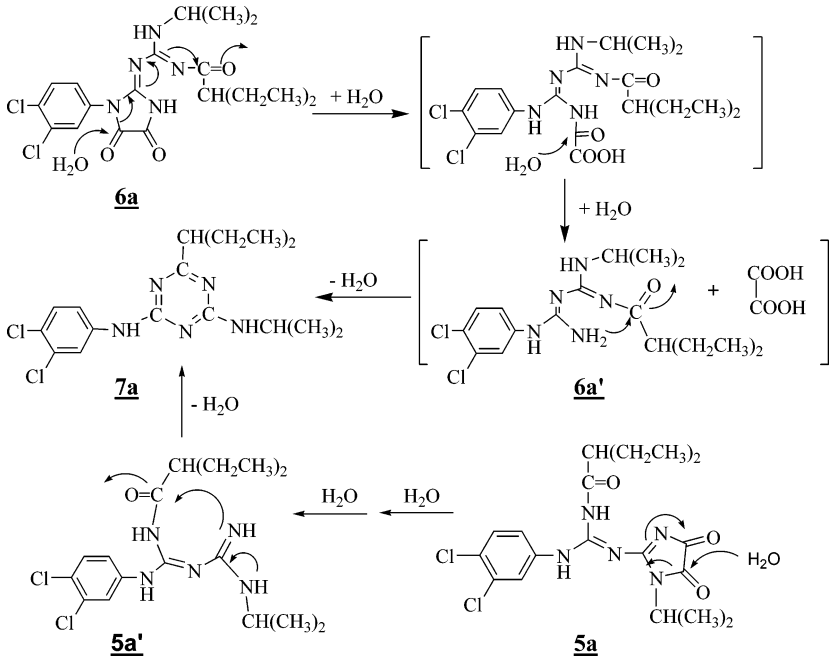


the blood stage parasites. One out of two monkeys that were treated with **5a** and one out of four monkeys that received compound **3** were cured. Other treated monkeys all showed a delay in relapse from 16 to 68 days, as compared to 9 days for untreated control animals (Table 3).

These preliminary monkey results are rather encouraging since monkeys were only treated for 3 days in this experiment, instead of 7 days for 8-aminoquinoline antimalarials, such as primaquine and tafenoquine, in the reported protocols.[19] Longer treatment with the test compounds may lead to a higher rate of cures. Compounds **3** and **5c** were again more active than **5a** and **6a** in the curative test, as in the prophylactic experiments (Table 2). The results indicated that compounds with better causal prophylactic activity also possess superior radical curative efficacy. Since no adverse side effects were observed in the monkeys treated with the test compounds at the level of 30 mg/kg × 3 days, further causal prophylactic and radical curative studies with treatment extended from 3 days to 7 consecutive days will be carried out.

In metabolic stability studies using mouse and human liver microsomal preparations, carboxamides **5a**−**c** were stable, with $t_{1/2} > \sim 60$ min, whereas the carboxamides **6a**−**c** were highly

Scheme 4. Mechanism of Chemical Transformation from Carboxamides **5a** and **6a** to Triazine **7a**



unstable, with a half-life ($t_{1/2}$) of less than 10 min (Table 1). The LC mass spectrum analysis of the metabolites indicated conversion of compounds **6a** to new substances, the molecular weight of which is 72 mass units (C_2O_3) less than that of the parent compound. The same conversion was also observed when the carboxamides were incubated in rat plasma. The observation suggests that carboxamides **6a**−**c** are the prodrugs of *s*-triazine **7a**−**c**, as shown in Scheme 3. The structure of the metabolite **7a** was confirmed by comparing it with authentic material of their mass spectra, LC retention times, and the spiking technique. The conversion of carboxamide **5a** to triazine **7a** was also demonstrated in rat PK studies, though at a slower rate. Likewise, carboxamides **6b** and **6c** were transformed to **7b** and **7c**, respectively. The rat plasma half-life of **6a** after intravenous injection (IV) was less than 10 min. The only metabolite that could be detected was compound **7a.** Detailed pharmacokinetics and metabolite identification of **5a**−**c** and **6a**−**c** will be published in a separate paper.

The mechanism of conversion of carboxamides to *s*-triazine is proposed as shown in Scheme 4. The mechanism involves hydrolysis of the imidazolidinedione ring of **6a** and **5a**, generating intermediates **5a′** and **6a′**. Cyclization of the intermediates and elimination of a water molecule yielded the *s*-triazine **7a**. Due to the electron-withdrawing effect of the dichlorophenyl ring, the carbonyl group of the imidazolidinedione ring of **6a** is more electron deficient than that of **5a**. Thus, the former is more susceptible to hydrolysis and form *s*-triazine **7a** at a faster rate than the latter.

The fact that transformation of **6a**−**c** to **7a**−**c** also took place when **6a**−**c** was incubated in buffer solution or plasma indicates that the conversion is a chemical transformation and not an enzymatic reaction. Furthermore, triazines **7a**−**c** are the only metabolites detected in the rat after oral or IM dosing with **6a**−**c**. The results suggest that **6a**−**c** may be the prodrugs of *s*-triazines **7a**−**c**. This possibility is substantiated by the observation that *s*-triazine **7a** also showed moderate causal prophylactic activity in the *P. yoelii* sporozoite-challenged mouse test (Table 1). Exploration of *s*-triazine derivatives as potential malaria prophylactic agents is currently in progress.

s-Triazine and dihydrotriazine derivatives have been reported to possess antimalarial activity in rodents.[20−22] However, the activities are generally confined to triazines with two unsubstituted free amino groups, an essential structure requirement for antifolate drugs. The triazine derivatives **7** are substituted 2,4-diaminotriazine derivatives, which showed no activity against both pyrimethamine-sensitive (D-6) and pyrimidine-resistant (W-2) clones of blood stage malarias *P. falciparum* in vitro and *P. berghei* in vivo, suggesting the causal prophylactic activity of *s*-triazines **7** in rodents is not due to inhibitory activity against dihydrofolate reductase, a key enzyme for parasite growth. Antimalarial activity of triazines **7** and the analogues will be discussed in a separate article.

Conclusion

A series of acid-stable carboxamide derivatives of 2-guanidinoimidazolidinediones (**5a**−**c** and **6a**−**c**) were prepared as potential malaria prophylactic and radical cure agents. Carboxamides **5a**−**c** are stable in human and mice liver microsomal preparations, but **6a**−**c** are unstable under the same test conditions with $t_{1/2} < 10$ min and were converted to *s*-triazine derivatives **7a**−**c**. Carboxamides **5a**−**c** also formed *s*-triazines but at a much slower rate than that of carboxamides **6a**−**c**. The conversion is a chemical transformation, not an enzyme-catalyzed reaction. The results suggest that the new carboxamides are prodrugs of *s*-triazine derivatives. Carboxamides **6a**−**c** are more active than carboxamides **5a**−**c** in the exoerythrocytic mouse model, but the latter showed superior causal prophylactic and radical curative activity in Rhesus monkeys. Therefore, the test results from mice infected with sporozoites of *P. yoelii* provide poor predictability of the efficacy in the Rhesus monkey test. As both amino groups of the triazines **7** are secondary, not primary, amines and that they showed no activity against the pyrimethamine-sensitive D-6 clone of *P. falciparum*, triazines **7** are not antifolate.

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Supporting Information Available: Analytical data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Trigg, P. I.; Kondrachine, A. V. The current global malaria situation. In *Malaria Parasite Biology, Pathogenesis and Protection*; Sherman, I. W., Ed.; ASM Press: Washington, D.C., 1998; Chapter 2, pp 11−22,

(2) World Health Organization. World malaria situation in 1994. *Wkly. Epidemiol. Rec.* **1997**, *72*, 269−76.

(3) Greenberg, A. E.; Lobel, H. O. Mortality from *Plasmodium falciparum*: malaria in travelers from the United States, 1959−1987. *Ann. Intern. Med.* **1990**, *113*, 326−27.

(4) White, N. J. Drug resistance in malaria. *Br. Med. Bull.* **1998**, *54*, 703−715.

(5) Nosten, F.; Ter Kuile, F.; Chongsuphajaisiddhi, T.; Luxemburger, C.; Webster, H. K.; Edstein, M.; Phaipun, L.; White, N. J. Mefloquine-resistant *falciparum* malaria on the Thai−Burmes border. *Lancet* **1982**, *337*, 1140−1143.

(6) Oduola, A. M.; Milhous, W. K.; Salako, L. A.; Walker, O.; Desjardins, R. E. Reduced in vitro susceptibility to mefloquine in West African isolates of *Plasmodium falciparum*. *Lancet* **1987**, *2*, 1304−1305.

(7) Carson, P. E.; Flanagan, C. I.; Ickes, C. E.; Alvin, A. S. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* **1956**, *124*, 484−485.

(8) Carson, P. E.; Hohl, R.; Nora, M. V.; Parkhurst, G. W.; Ahmad, T.; Scanlan, S.; Frischer, H. Toxicology of the 8-aminoquinolines and generic factors associated with their toxicity in man. *Bull. W. H. O.* **1981**, *59*, 427−437.

(9) Schmidt, L. H.; Frank, R.; Genther, C. S.; Rossan, R. N.; Squires, W. I. The characteristics of untreated sporozoite-induced and trophozoite-induced infections. *Am. J. Trop. Med. Hyg.* **1982**, *31*, 612−645.

(10) Schmidt, L. H.; Frank, R.; Genther, C. S.; Rossan, R. N.; Squires, II, W. Responses of Sporozoite-induced and trophozoite-induced infections to standard antimalarials drugs. *Am. J. Trop. Med. Hyg.* **1982**, *31*, 646−665.

(11) Puri, S. K.; Dutta, G. P. Quinoline esters as potential antimalarial drugs: effect on relapses of *Plasmodium cynomolgi* infections in monkeys. *Trans. R. Soc. Trop. Med. Hyg.* **1990**, *84*, 759−60.

(12) Peters, W. The evolution of tafenoquine−antimalarial for a new millennium? *J. R. Soc. Med.* **1999**, *92*, 345−52.

(13) Walsh, D. S.; Wilairatana, P.; Tang, D. B.; Heppner, D. G., Jr.; Brewer, T. G.; Krudsood, S.; Silachamroon, U.; Phumratanaprapin, W.; Siriyanonda, D.; Looareesuwan, S. Randomized trial of 3-dose regimens of tafenoquine (WR238605) versus low-dose primaquine for preventing *Plasmodium vivax* malaria relapse. *Clin. Infect. Dis.* **2004**, *39*, 1095−103.

(14) Walsh, D. S.; Eamsila, C.; Sasiprapha, T.; Sangkharomya, S.; Khaewsathien, P.; Supakalin, P.; Tang, D. B.; Jarasrumgsichol, P.; Cherdchu, C.; Edstein, M. D.; Rieckmann, K. H.; Brewer, T. G. Efficacy of Monthly Tafenoquine for Prophylaxis of Plasmodium vivax and Multidrug-resistant P. falciparum Malaria. *J. Infect. Dis.* **2004**, *190*, 1456−63.

(15) Corcoran, K. D.; Hansukjariya, P.; Sattabongkot, J.; Ngampochjana, M.; Edstein, M. D.; Smith, C. D.; Shanks, G. D.; and Milhous. W. K. Causal prophylactic and radical curative activity of WR182393 (a guanylhydrazone) against *Plasmodium cynomolgi* in *Macaca mulatta*. *Am. J. Trop. Med. Hyg.* **1993**, *49*, 473−477.

(16) Guan, J.; Zhang, Q.; Gettayacamin, M.; Karle, J. M.; Ditusa, C. A.; Milhous, W. K.; Skillman, D. R.; Lin, A. J. Structure identification and prophylactic antimalarial efficacy of 2-guanidinoimidazolidinedione derivatives. *Bioorg. Med. Chem.*, **2005**, *13*, 699−704.

(17) Zhang, Q.; Guan, J.; Sacci, J.; Ager, A.; Ellis, W. Y.; Milhous, W. K.; Kyle, D. E.; Lin, A. J. Unambiguous synthesis and prophylactic antimalarial activities of imidazolidinedione derivatives. *J. Med. Chem.*, **2005**, *48*, 6472−6481.

(18) Lin, A. J.; Zhang, Q.; Guan, J.; Milhous, W. K. 2-Guanidinylimidazolidinedione Compounds of Making and Using Thereof. U.S. Patent Number 7,101,902, September 5, 2006.

(19) Schmidt, L. H.; Alexander, S.; Allen, L.; Jane Rasco, J. Comparison of the curative antimalarial activities and toxicities of primaquine and its D and L Isomers. *Antimicrob. Agents Chemother.* **1977**, *12*, 51−60.

(20) Smith, C. C.; Ihrig, J.; Menne, R. Antimalarial activity and metabolism of biguanides. *Am. J. Trop. Med. Hyg.* **1961**, *10*, 694−703.

(21) Watkins, W. M.; Sixsmith, D. G.; Chulay, J. D. The activity of proguanil and its metabolites, cycloguanil and *p*-chlorophenylbiguanide, against *Plasmodium falciparum* in vitro. *Ann. Trop. Med. Parasitol.* **1984**, *78*, 273−278.

(22) Kinyanjui, S. M.; Mberu, E. K.; Winstanley, P. A.; Jacobus, D. P.; Watkins, W. M. The antimalarials triazine WR99210 and the prodrug PS-15: folate reversal of in vitro activity against *Plasmodium falciparum* and a non-antifolate mode of action of the prodrug. *Am. J. Trop. Med. Hyg.* **1999**, *60*, 943−947.

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