

In vitro and in vivo characterization of the antimalarial lead compound SSJ-183 in *Plasmodium* models

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Abstract: The objective of this work was to characterize the in vitro (*Plasmodium falciparum*) and in vivo (*Plasmodium berghei*) activity profile of the recently discovered lead compound SSJ-183. The molecule showed in vitro a fast and strong inhibitory effect on growth of all *P. falciparum* blood stages, with a tendency to a more pronounced stage-specific action on ring forms at low concentrations. Furthermore, the compound appeared to be equally efficacious on drug-resistant and drug-sensitive parasite strains. In vivo, SSJ-183 showed a rapid onset of action, comparable to that seen for the antimalarial drug artesunate. SSJ-183 exhibited a half-life of about 10 hours and no significant differences in absorption or exposure between noninfected and infected mice. SSJ-183 appears to be a promising new lead compound with an attractive antimalarial profile.

Keywords: antimalarial studies, cross-resistance, stage-specificity, *Plasmodium falciparum*

Introduction

In 2010, 216 million people were infected with malaria, and an estimated 665,000 were killed by this devastating parasitic disease, according to World Health Organization (WHO) reports.¹ Of the nearly 1,400 drugs registered worldwide in the last quarter of the 20th century, only four were antimalarials.² The high costs of available antimalarials and the spread of resistance dramatically minimize the choice of effective medicines in endemic countries; this situation demands renewed efforts toward development of new therapeutic agents.

The recently discovered SSJ-183 is a benzo[α]phenoxazine-based antimalarial (Figure 1).³ Two-dimensional gel electrophoresis studies investigating the mechanism of action of this compound were performed with the rodent malaria parasite SSJ-183 *Plasmodium berghei* and indicated a significant change of the expression levels of nine proteins that represent potential therapeutic targets.⁴ The reported in vitro half-maximal inhibitory concentration (IC_{50}) values of 3 ng/mL against the parasite strain K1 and 22,000 ng/mL against L6 myoblasts in a cytotoxicity test indicate a specific antimalarial potency of the compound (selectivity index of >7,000).³ The selectivity was further supported by the $\geq 1,000$ -fold higher IC_{50} values of SSJ-183 against three other protozoal parasites (*Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi* and *Leishmania donovani*) as compared with K1. In vivo data were obtained from NMRI mice infected with the *P. berghei* ANKA strain; the infected mice were cured by oral doses of 100 mg/kg given for 3 consecutive days (3×100 mg/kg).³ Three \times oral doses of 30 mg/kg cured 80% of the mice, whereas 3×10 mg/kg was entirely ineffective.

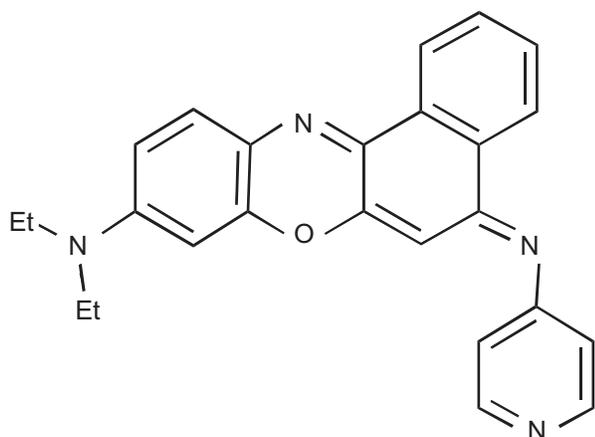


Figure 1 Molecular structure of SSJ-183.

The objectives of the present study were to further assess the potential of SSJ-183 for malaria chemotherapy. Such information is needed to assess whether the molecule could fit into the target product profiles of, for example, the nonprofit organization Medicines for Malaria Venture (MMV).⁵

Materials and methods

Chemicals and materials

Antimalarial compounds were dissolved in dimethyl sulfoxide (DMSO; 10 mg/mL) (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) and stored at 4°C. The following compounds were tested: pyrimethamine, proguanil, and mefloquine (F Hoffmann-La Roche Ltd, Basel, Switzerland); chloroquine, amodiaquine and artesunate (Sigma-Aldrich Chemie GmbH); SSJ-183 (M Ihara, Hoshi University, Japan); artemisinin (Chongqing Holley Wuling Mountain Pharmaceuticals Co, Ltd, Huawu, People's Republic of China); piperaquine (Sigma Tau, Pomezia, Italy); pyronaridine (AvaChem Scientific, San Antonio, TX, USA); and atovaquone (GlaxoSmithKline, Tres Cantos, Spain). [³H]hypoxanthine and nonradioactive hypoxanthine were purchased from ANAWA Biomedical Services and Products (Zürich, Switzerland) and Fluka Analytical, (Sigma-Aldrich Chemie GmbH), respectively.

Parasite cultivation

The drug-sensitive *P. falciparum* strain NF54 (airport strain from The Netherlands) was provided by F. Hoffmann-La Roche Ltd; the chloroquine (CQ)- and pyrimethamine-resistant strain K1 (Thailand) was obtained from the Malaria Research and Reference Reagent Resource Center (MR4; Manassas, VA, USA) (MRA-159). Apart from these two, five additional isolates were used in cross-resistance tests,

namely 7G8 (Brazil), TM90C2A (Thailand), D6 (Sierra Leone), W2 (Indochina) and V1/S (Vietnam), all from MR4 (MRA-154, -202, -285, -157, -176). All strains except for D6 share CQ- and pyrimethamine-resistance with K1; TM90C2A is additionally resistant to mefloquine. No resistance is known for D6. The parasites were cultivated as has been described previously.⁶

[³H]hypoxanthine incorporation assay

Parasite growth in the presence of antimalarial compounds was assessed using the [³H]hypoxanthine incorporation assay and expressed by IC₅₀ values.⁶ The total incubation time per assay was 72 hours; radioactive hypoxanthine was added for the last 24 hours. The respective IC₅₀ values in ng/mL against unsynchronized NF54 parasites were 3.0 (pyrimethamine), 440 (proguanil),⁶ 4.1 (mefloquine), 1.5 (amodiaquine), 3.5 (artesunate), 3.2 (SSJ-183), 3.0 (artemisinin), 7.8 (piperaquine), 2.6 (pyronaridine) and 0.38 (atovaquone).⁶

In vitro assessment of stage-specific activity

Cultures were synchronized twice with 5% D-sorbitol as described previously.^{7,8} The second treatment was 6–8 hours after the first. This procedure provided a parasite culture containing ≥80% young trophozoites (up to 20 hours old). The same procedure, but with another 16 hours of cultivation after the second sorbitol treatment, was carried out separately to obtain early schizonts (up to 36 hours old).

To obtain ring forms, the two synchronizing treatments were divided by a cultivation period of 34 hours rather than 6–8 hours, yielding a parasite culture with >80% rings (up to 6 hours old). Two 96-well microtiter plates for each of the three synchronous stages were incubated with two-fold serial dilutions of SSJ-183 and pyrimethamine, one for 6 hours and the second for 24 hours (final concentration ranges of both antimalarials were 250–7.81 ng/mL). For a concentration of 250 ng/mL the SSJ-183 stock solution (10 mg/mL) was first diluted 1/50 in DMSO, followed by a 1/100 dilution and another 1/2 dilution in the hypoxanthine-free version of the medium as described by Maerki et al.⁹ The DMSO concentration in the experiments (<0.5%) had no inhibitory effect on parasite cultures. The washing procedures and further processing of the plates were carried out according to published procedures.^{8,9}

In vitro drug–drug interactions

In vitro interactions of 1:1, 1:3 and 3:1 ratios of SSJ-183 with amodiaquine, artemisinin, mefloquine, piperaquine, or

pyronaridine were investigated using a modified fixed-ratio isobologram method and the NF54 and K1 parasite strain.^{6,10} As previously recommended, interactions were classified as synergistic or antagonistic with sums of fractional inhibitory concentrations (Σ FICs) of ≤ 0.8 and > 1.4 , respectively, with any value in between indicating no interaction.⁶ Cutoff ranges were determined by mixing the same drug at various ratios and accounting for experimental variation.

In vitro cross-resistance with various strains of *P. falciparum*

The sensitivity of the *P. falciparum* isolates NF54, K1, W2, 7G8, TM90C2A, D6, and V1/S to SSJ-183 was assessed by comparison of the respective IC_{50} values. The ratio between the maximal and minimal obtained value among the results of all tested strains was taken as a means to assess possible cross-resistance. SSJ-183 stock solution (10 mg/mL) was directly diluted in the hypoxanthine-free medium, resulting in an IC_{50} of 21 ng/mL against unsynchronized NF54 parasites. As mentioned previously, in the experiments where stage-specific action was assessed, the SSJ-183 stock solution (10 mg/mL) was first diluted 1/50 in DMSO, which resulted in an IC_{50} value that was ~ 7 times lower (3.2 ng/mL).

Statistical analysis

The data for the pharmacodynamic profile of SSJ-183 are expressed as the mean \pm standard deviation (SD) of 3–4 independent experiments. To evaluate statistical significance of stage-dependent differences in drug-sensitivity at a level of 5%, a one-way ANOVA followed by the Tukey Honestly Significant Difference (HSD) test was performed using the software available online from VassarStats (<http://www.vassarstats.net/>).

Similarly, the mean Σ FIC values are the means of 3 independent experiments \pm SD. Cross-resistance data were based on the averaged results of 2–3 independent experiments.

In vivo onset of action

In vivo efficacy was conducted as previously described using *P. berghei*-infected mice.¹¹ SSJ-183 and the comparator drug artesunate were dissolved or suspended in a vehicle consisting of 0.5% (w/v) hydroxypropyl methyl cellulose, 0.5% (v/v) benzyl alcohol, 0.4% (v/v) Tween[®] 80 (Sigma-Aldrich, St Louis, MO, USA), and 0.9% (w/v) sodium chloride in water. The onset of action was determined after a single oral dose of 100 mg/kg of compounds to five female NMRI mice (20–22 g) on day 3 postinfection, resulting in a high initial parasitemia ($\sim 40\%$) to allow the onset of action

to be assessed. The reduction in parasitemia and the time of recrudescence was assessed by daily blood analysis (standard flow cytometry techniques) for 7 days followed by intermittent assessment up to 11 days.

Pharmacokinetic properties

The pharmacokinetics properties of SSJ-183 maleate were studied in parallel groups of mice, including noninfected NMRI mice and *P. berghei*-infected NMRI mice with dosing on day 1 postinfection. SSJ-183 was administered at a nominal dose of 20 mg/kg by oral gavage of a 2 mg/mL suspension (10 mL/kg) prepared in an aqueous vehicle containing 0.5% (w/v) hydroxypropyl methyl cellulose, 0.5% (v/v) benzyl alcohol and 0.4% (v/v) Tween[®] 80. Blood samples were collected from the tail vein at 0.5, 1, 2, 4, 8, 24, 32, and 48 hours (n=3 mice per time point). Blood was collected into calibrated and heparinized capillaries, transferred to Eppendorf tubes, placed on ice immediately and centrifuged (5,000 rpm for 5 min) as soon as possible (within 15 minutes of collection). The supernatant plasma was removed and stored deep-frozen (-70°C) in the dark until analysis.

Plasma concentrations of SSJ-183 and its metabolites, N-deethyl SSJ-183 and bis-N,N-deethyl SSJ-183, were determined by liquid chromatography-mass spectrometry. Calibration standards were prepared by spiking blank mouse plasma with SSJ-183 and each of the metabolites. Following the addition of internal standard (diazepam), proteins were precipitated by the addition of acetonitrile at a ratio of 3:1 acetonitrile:plasma. Samples were vortexed, centrifuged, and the supernatant (5 μL) injected directly onto the column (Supelco Ascentis[®] Express RP-Amide column [Sigma-Aldrich], 2.7 μm particle size, 50 \times 2.1 mm in diameter equipped with a Phenomenex Security Guard cartridge with a Synergi Polar RP insert [Phenomenex, Torrance, CA, USA] and maintained at 40 $^{\circ}\text{C}$). Chromatography was conducted using a Waters Acquity UPLC[®] (Waters Corporation, Milford, MA, USA) with a mobile phase of 0.005 M ammonium formate in water (A) and 0.005 M ammonium formate in methanol (B), delivered at 0.4 mL/min, and mixed using a linear gradient from 10 to 95% B over 3.5 min, followed by a re-equilibration for 0.5 min prior to the next injection. Detection was conducted using a Waters Xevo TQ (Waters Corporation) triple quadrupole instrument operated in positive mode electrospray ionization with a capillary voltage and detector multiplier voltage of 3.5 kV and 500 V, respectively, and source block and desolvation temperature of 120 $^{\circ}\text{C}$ and 650 $^{\circ}\text{C}$; the analyte elution was monitored using multiple reaction monitoring (MRM). Metabolite identity

was confirmed by comparing the MS/MS spectrum and chromatographic retention characteristics to those for the authentic standards. Plasma concentrations of the analytes were quantified by an internal standard method using the system software, Quanlynx 4.1 (Micromass, Fort Myers, FL, USA). Accuracy, precision, and linearity for each of the analytes were assessed over the range of 0.5–10,000 ng/mL; the analytical lower limit of quantitation (LLQ) was typically 0.5 ng/mL for SSJ-183, and 1 ng/mL for N-deethyl SSJ-183 and bis-N,N-deethyl SSJ-183. Plasma concentration versus time profiles were analyzed by noncompartmental methods (WinNonlin v 5.2.1, Pharsight Corp, Mountain View, CA, USA), using mean concentrations at each time point.

Results

In vitro assessment of stage-specific activity

The time- and concentration-related stage-specificity of SSJ-183 was assessed in vitro using the [³H]hypoxanthine incorporation assay. Pyrimethamine served as the control drug, and similar to earlier findings,⁸ it was found to be ineffective against ring and trophozoite stages (data not shown). The inhibitory effects on parasite growth at 6 and 24 hours

of incubation with SSJ-183 at six different concentrations (250, 125, 62.5, 31.3, 15.6, and 7.81 ng/mL) are shown in Figure 2. SSJ-183 was highly active and impaired growth of all three intraerythrocytic parasite stages by >99% at the highest concentration (250 ng/mL) with both incubation times, and at the intermediate concentration (62.5 ng/mL) after 24 hours. After 6 hours at the lowest concentration (7.81 ng/mL), the ring forms were significantly more susceptible than schizonts ($P<0.05$), with a residual growth of 35%, followed by trophozoites (62% growth), and schizonts (100% growth). The same tendency was observed at the intermediate concentration (62.5 ng/mL), where rings and trophozoites showed <3%, and schizonts 32%, growth. Similarly, the schizonts were significantly ($P<0.05$) less susceptible than both of the other two stages at concentrations of 31.3 and 15.6 ng/mL. After 24 hours of incubation, the rings were again found to be the most susceptible parasite stage for all applied concentrations ≤ 31.3 ng/mL, although this finding was not statistically significant.

In vitro drug–drug interactions

SSJ-183 was combined with a panel of five currently used antimalarials to assess potential drug interactions at three

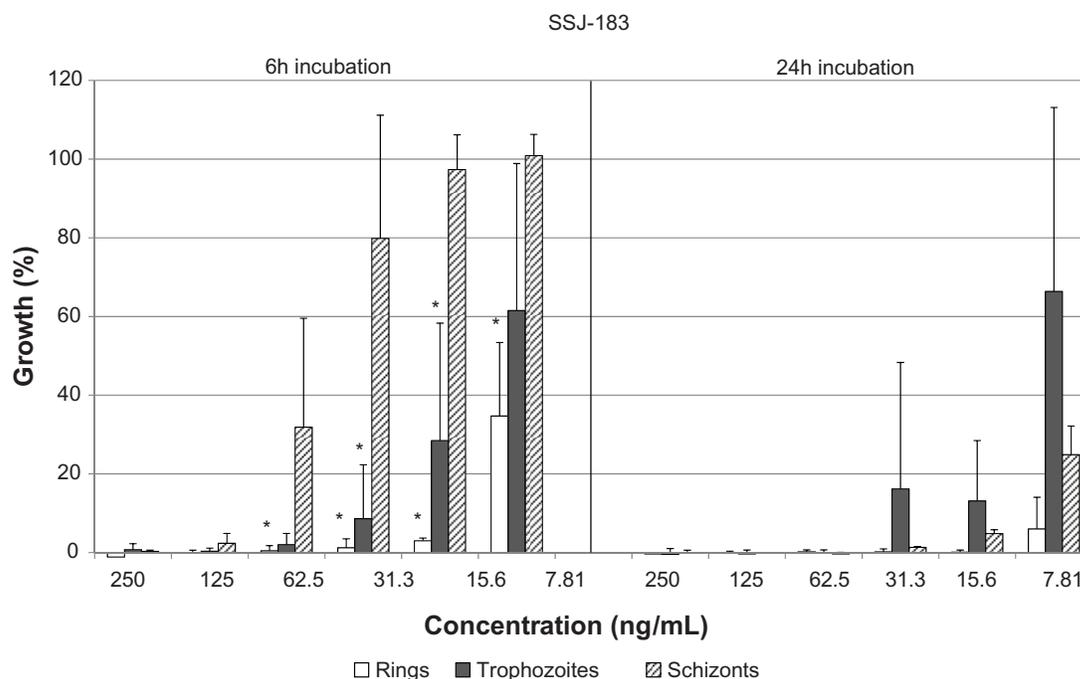


Figure 2 Stage-dependent effects of SSJ-183 on [³H]hypoxanthine incorporation in synchronous cultures of *Plasmodium falciparum* strain NF54.

Notes: Cultures were exposed to seven different concentrations of the compound for 6 or 24 hours. After removal of SSJ-183, parasites were incubated for another 24 hours in the presence of [³H]hypoxanthine. Compound effects are expressed as the percentage of growth of the respective development stage relative to an untreated control. The open bar is the ring stage, the filled bar is the trophozoite stage and the hatched bar is the schizont stage. Each bar represents the mean \pm SD of $n=3-4$ independent experiments. Significant ($*P<0.05$) differences between ring- and schizont-stages or trophozoites- and schizont-stages were determined by a one-way ANOVA with Tukey's Honestly Significant Difference (HSD) test.

Abbreviations: SD, standard deviation; n, number; h, hours.

different ratios (1:3, 1:1, and 3:1) using the fixed-ratio isobologram method.¹⁰ The results are summarized in Table 1. The previously described synergism of the control combination of atovaquone + proguanil was confirmed by our data (mean Σ FICs in the range 0.23–0.31).⁶ We tested SSJ-183 in combination with artemisinin. The mean range of the Σ FICs (0.69–0.79) indicated synergism. Combinations with mefloquine or piperazine showed additive action (mean Σ FICs for K1/NF54 were 1.2/1.1 and 1.4/1.3.), and amodiaquine or pyronaridine combinations appeared antagonistic (mean Σ FICs for K1/NF54 were 1.5/1.4 and 1.7/1.7).

In vitro cross-resistance with various strains of *P. falciparum*

SSJ-183 was found to potently inhibit growth of all seven strains tested (IC_{50} values from 17 to 50 ng/mL) irrespective of the drug-sensitivity status of the parasites (Table 2). In contrast, five out of seven strains showed considerable resistance against the control drugs chloroquine and pyrimethamine (IC_{50} values 5.7–237 ng/mL and 1.3–5,456 ng/mL, respectively). Notably, the activity of SSJ-183 and its lack of cross-resistance was comparable to that of the clinically used comparator drug artesunate for every strain tested (IC_{50} values for artesunate: 2.1–5.0 ng/mL).

In vivo onset of action

Single oral doses of 100 mg/kg SSJ-183 or artesunate were administered to *P. berghei*-infected mice on day 3 postinfection. SSJ-183 had a rapid onset of action against *P. berghei* comparable to that seen for artesunate (Figure 3). Recrudescence for both compounds was also comparable,

becoming evident by days 7–10 postinfection. The single oral dose where 90% reduction of parasitemia compared to untreated control animals was achieved (ED90) was found to be 20 mg/kg (data not shown).

Pharmacokinetic properties

The plasma concentration versus time profiles for noninfected and infected mice are shown in Figure 4, and the corresponding pharmacokinetic data are presented in Table 3. Following oral administration of SSJ-183 at the ED90 (20 mg/kg), absorption was relatively fast with maximum plasma concentrations (C_{max}) observed 1 hour postdose in both noninfected and infected groups. The terminal elimination phase was prolonged and the terminal half-life was approximately 10 hours in both groups. There was no significant difference in absorption or exposure between the two groups.

The metabolites N-deethyl SSJ-183 and bis-N,N-deethyl SSJ-183 showed similar in vitro potency when compared to SSJ-183 (data not shown) and were detected in the plasma of all mice throughout the postdose sampling period, with maximum concentrations observed 4–8 hours postdose, followed by prolonged terminal elimination phases. As for the parent compound, differences were minimal between the noninfected versus infected groups.

Discussion

The susceptibility of ring forms, trophozoites, and schizonts of *P. falciparum* to the lead compound SSJ-183 were determined. At 24 hours incubation time and the highest concentration, SSJ-183 impaired the growth of all stages by >99%. For most of the lower concentrations, the ring

Table 1 In vitro interaction of drug combinations against two *Plasmodium falciparum* strains

Drug combination	Σ FIC ^a						Mean Σ FIC		Interaction
	K1			NF54			K1	NF54	
	1:3	1:1	3:1	1:3	1:1	3:1			
Atovaquone + proguanil	0.28 ± 0.02	0.22 ± 0.02	0.20 ± 0.02	0.37 ± 0.08	0.29 ± 0.06	0.26 ± 0.31	0.23 ± 0.02	0.31 ± 0.07	Synergism
SSJ-183 + artemisinin	0.67 ± 0.06	0.73 ± 0.15	0.97 ± 0.31	0.60 ± 0.10	0.67 ± 0.15	0.80 ± 0.17	0.79 ± 0.16	0.69 ± 0.10	Synergism
SSJ-183 + mefloquine	1.2 ± 0.5	1.2 ± 0.4	1.2 ± 0.6	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.2	1.2 ± 0.0	1.1 ± 0.0	Additive action
SSJ-183 + piperazine	1.5 ± 0.3	1.5 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	Additive action
SSJ-183 + amodiaquine	1.7 ± 0.2	1.5 ± 0.2	1.4 ± 0.3	1.6 ± 0.3	1.7 ± 0.3	1.0 ± 0.3	1.5 ± 0.2	1.4 ± 0.4	Antagonism
SSJ-183 + pyronaridine	1.6 ± 0.6	1.8 ± 0.5	1.5 ± 0.3	1.7 ± 0.2	1.8 ± 0.6	1.4 ± 0.4	1.7 ± 0.1	1.7 ± 0.2	Antagonism

Notes: ^aValues are the means and standard deviations from three or more experiments.

Abbreviations: Σ FIC, sum of fractional inhibitory concentration; ND, not determined.

Table 2 In vitro cross-resistance experiments with various sensitive and resistant *Plasmodium falciparum* strains (IC_{50} values are the mean of 2 to 3 independent experiments)

Isolate	Origin	Resistance	IC_{50} (ng/mL) SSJ-183*	IC_{50} (ng/mL) chloroquine ^a	IC_{50} (ng/mL) pyrimethamine ^a	IC_{50} (ng/mL) artesunate
NF54	Airport, NL	–	21	5.7	4.5	3.5
K1	Thailand	CQ, PYR	23	157	2,521	2.6
W2	Indochina	CQ, PYR	24	169	3,463	2.9
7G8	Brazil	PYR	17	71	2,607	2.1
TM90C2A	Thailand	CQ, PYR, MQ	50	90	4,787	4.1
D6	Sierra Leone	–	39	8.3	1.3	5.0
VI/S	Vietnam	CQ, PYR	21	237	5,456	3.1
Maximum IC_{50}			50	237	5,456	5.0
Minimum IC_{50}			17	5.7	1.3	2.1
Maximum/minimum			2.9-fold	42-fold	4200-fold	2.4-fold

Notes: *In this experiment, SSJ-183 stock solution (10 mg/mL) was directly diluted in the hypoxanthine-free medium, resulting, for example, in an IC_{50} against NF54 parasites that is $\sim 7\times$ higher compared with the value obtained when the SSJ-183 stock solution was first diluted 1/50 in DMSO (3.2 ng/mL). ^aAdapted with permission from Brunner R, Aissaoui H, Boss C, et al. Identification of a new chemical class of antimalarials. *J Infect Dis.* 2012;206(5):735–743.¹⁷

Abbreviations: IC_{50} , Half-maximal inhibitory concentration; DMSO, dimethyl sulfoxide; NL, The Netherlands.

forms showed the highest susceptibility. This observation is similar to findings reported for methylene blue, a molecule related to SSJ-183.³ After 6 hours of incubation with SSJ-183, schizont growth was affected significantly less than that of both rings and trophozoites.

In the *P. berghei* mouse model, a single oral dose of 100 mg/kg of SSJ-183 demonstrated a prompt onset of action, which was comparable to that of the artemisinin derivative artemether.¹¹ This is significant, as the artemisinin derivatives are considered to be the most potent and fast-acting class of antimalarials currently available. Following oral administration to mice, SSJ-183 also demonstrated a long half-life, with plasma concentrations maintained above the IC_{50} for longer than 48 hours at the 20 mg/kg oral dose.

Drug combinations are an essential strategy to prevent or delay the emergence and spread of resistance. The current

first-line treatments for both uncomplicated and severe malaria, as recommended by the World Health Organization (WHO), are combination therapies. SSJ-183 and artemisinin showed synergistic interactions at the 1:3 and 1:1 ratios (mean $\Sigma FICs$, 0.60–0.73), whereas the combination ratio of 3:1 was additive (mean $\Sigma FICs$, 0.80–0.97). Although the synergistic action of SSJ-183/artemisinin was less pronounced compared with atovaquone/proguanil, the data fits well with earlier findings describing synergistic interactions between artemisinin derivatives and methylene blue.¹² The artemisinins are administered as artemisinin-based combination therapies (ACTs) and are the recommended first-line therapy to clear *P. falciparum* infections. Although the clinical and parasitological efficacy of ACTs remains high in most settings,¹ prolonged parasite clearance times have been reported in Western Cambodia.^{13–15}

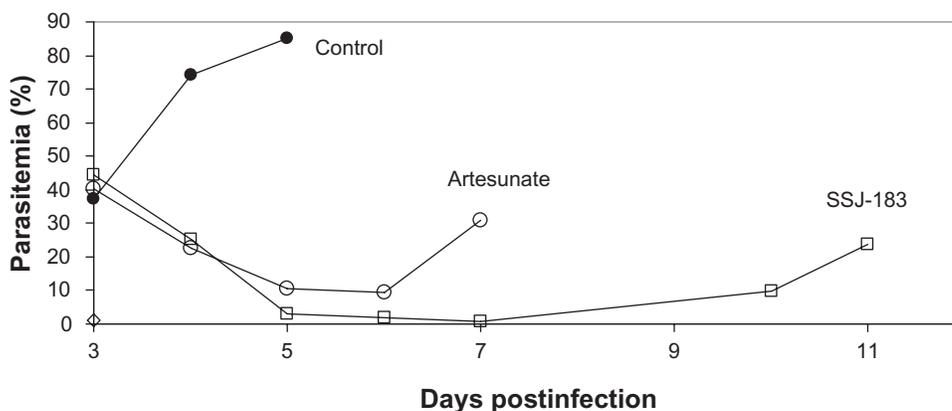


Figure 3 In vivo efficacy in *Plasmodium berghei*-infected mice: onset of action and recrudescence for control (●), artesunate (○) and SSJ-183 (□) following a single oral dose of 100 mg/kg on day 3 postinfection (n=5 mice for each).

Abbreviation: n, number.

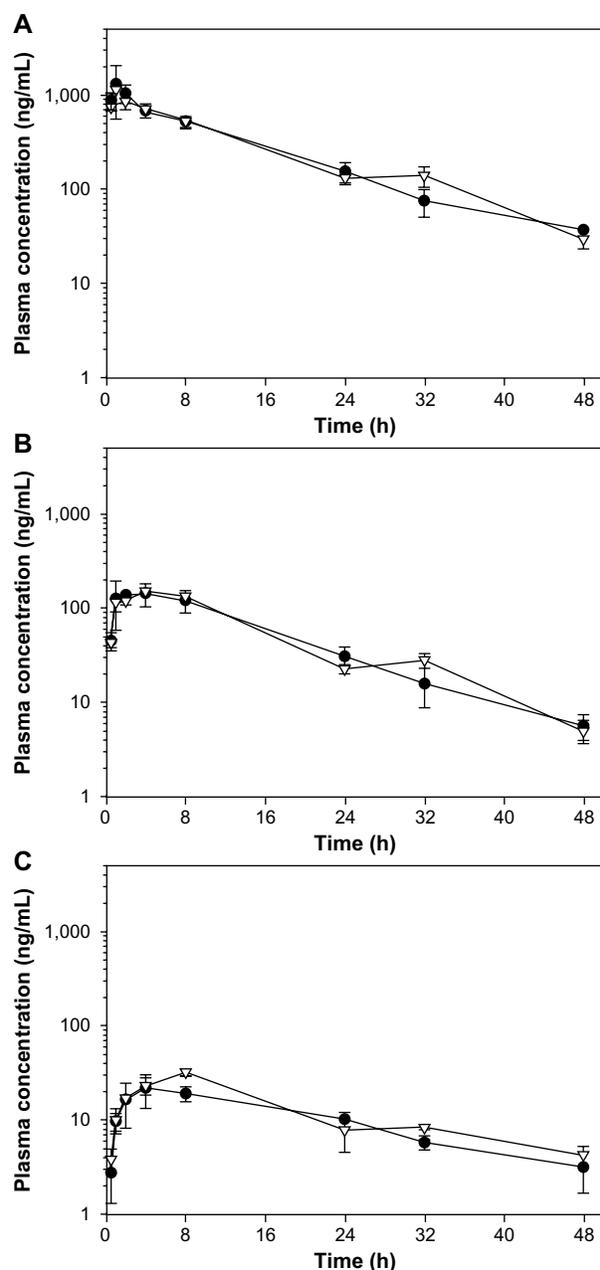


Figure 4 Plasma concentration versus time profiles for (A) SSJ-183, (B) N-deethyl SSJ-183, and (C) bis-N,N-deethyl SSJ-183 following oral administration of SSJ-183 (20 mg/kg) to noninfected (filled circles) and *Plasmodium berghei*-infected (open triangles) NMRI mice.

Note: Data represents the mean \pm SD for $n=3$ mice per time point.

Abbreviations: SD, standard deviation; h, hours.

Laboratory studies and mathematical modeling suggest that these changes in clearance time of parasites mainly result from decreased susceptibility of ring-stage parasites. Therefore, if the partner drug of an ACT acts specifically on the trophozoite or schizont stage, as is the case for most of the drugs, the artemisinin portion of the combination is virtually administered as a monotherapy in respect to the

Table 3 Pharmacokinetic parameters for SSJ-183, N-deethyl SSJ-183 and bis-N,N-deethyl SSJ-183 after oral administration (20 mg/kg) of SSJ-183 to noninfected and *Plasmodium berghei*-infected NMRI mice

Parameter	SSJ-183	N-deethyl SSJ-183	Bis-N,N-deethyl SSJ-183
Noninfected mice			
$T_{1/2}$ (h)	10.3	9.0	15.2
C_{max} (ng/mL)	1,297.8	139.6	21.1
T_{max} (h)	1	4	4
AUC_{0-inf} (h·ng/mL)	13,893	2,569	556
<i>P. berghei</i> -infected mice			
$T_{1/2}$ (h)	10.1	8.8	14.3
C_{max} (ng/mL)	1,108.1	147.1	31.1
T_{max} (h)	1	4	8
AUC_{0-inf} (h·ng/mL)	13,847	2,715	715

Abbreviations: $T_{1/2}$, half-time; C_{max} , maximum concentration; T_{max} , maximum time; AUC_{0-inf} , area under the curve from time 0 to infinity; h, hours; *P. berghei*, *Plasmodium berghei*.

parasiticidal effect on ring-forms. The tendency of SSJ-183 to act at low concentrations specifically on rings might thus help to protect this more vulnerable phase in the life-saving treatment with ACTs.

Assessing cross-resistance against antimalarials is a challenge, as there are problems to concisely define drug-resistant malaria, especially in vitro.¹⁶ To account for the baseline assay-to-assay variation, artesunate served as a reference in our studies. The finding that SSJ-183 inhibited parasite growth of all tested *P. falciparum* strains in an IC_{50} range similar to that of artesunate irrespective of the sensitivity status of the strain suggests that cross-resistance may not be a concern for SSJ-183.

In conclusion, SSJ-183 has an attractive antimalarial profile and could possibly be combined with a variety of different drugs without losing its efficacy. The activity of SSJ-183 remains unaffected by cross-resistance against several antimalarials, and it shows a reasonable half-life and no significant differences in absorption or exposure in non-infected versus infected animals. These findings support the new molecule as a promising candidate for further profiling studies, such as the verification of its safety and its ability to be able to reduce transmission and to prevent relapse of dormant parasite forms.

Acknowledgments

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Disclosure

The authors declare no conflicts of interest in this study.

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