SC83288 is a clinical development candidate for the treatment of severe malaria

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Severe malaria is a life-threatening complication of an infection with the protozoan parasite Plasmodium falciparum, which requires immediate treatment. Safety and efficacy concerns with currently used drugs accentuate the need for new chemotherapeutic options against severe malaria. Here we describe a medicinal chemistry program starting from amicarbalide that led to two compounds with optimized pharmacological and antiparasitic properties. SC81458 and the clinical development candidate, SC83288, are fast-acting compounds that can cure a P. falciparum infection in a humanized NOD/SCID mouse model system. Detailed preclinical pharmacokinetic and toxicological studies reveal no observable drawbacks. Ultra-deep sequencing of resistant parasites identifies the sarco/endoplasmic reticulum Ca2+ transporting PfATP6 as a putative determinant of resistance to SC81458 and SC83288. Features, such as fast parasite killing, good safety margin, a potentially novel mode of action and a distinct chemotype support the clinical development of SC83288, as an intravenous application for the treatment of severe malaria.
The decline in global malaria mortality rates by 48% over the previous decade has raised hopes of radical malaria control. However, the burden of malaria is still high. There were 213 million cases of malaria and 438,000 deaths in 2015 alone, with 3.2 billion people at risk. Moreover, much of the success gained against malaria has been owed to the excellent therapeutic efficacy of artemisinin derivatives and their partner drugs, which together as an artemisinin-based combination therapy (ACT) have formed the backbone of malaria control since 2005 (ref. 2). However, the clinical efficacy of ACT is under attack. Plasmodium falciparum strains have emerged that display a delayed and possibly reduced responsiveness to ACT. Such early stage ACT resistant parasites have recently spread from their place of origin at the Thai/Cambodian border across all of Southeast Asia and run the risk of spreading further into Asia and beyond. The consequences would be devastating since alternatives to ACTs are not readily available.

The evolving lack of chemotherapeutic options against malaria accentuates the need for new antimalarial drugs. Such novel drugs should exploit novel molecular targets and have distinct chemical structures to protect them from cross-resistance mechanisms. Several candidates that meet these criteria are in the development pipeline. However, many more would be needed to compensate for the high attrition rate expected during the clinical trials and to build up a stock of reserve antimalarial drugs that can quickly replace first-line drugs should they fail.

In addition to novel drugs for the treatment of uncomplicated malaria, there is an equally urgent need for a new generation of drugs for the treatment of severe malaria. Severe malaria is a life-threatening condition that inevitably leads to the death if not treated immediately. The leading symptoms of severe malaria include impaired consciousness, vital organ dysfunction, hyperparasitaemia and the inability to take oral medicine. Severe malaria is currently treated with intravenous (i.v.) or intramuscular artesunate for at least 24 h or, if parenteral artesunate or artemether are not available, with i.v. quinine. However, in addition to the resistance problem outlined above, there are concerns regarding the safety of both treatment regimens. i.v. artesunate has recently been associated with delayed haemolysis in 7–21% of the treated patients and quinine is inactive. We therefore explored a wider range of substituents including carbonyl-amino, amino-sulfonyl and amino-carbonyl groups, but decided to focus on 4-sulfonamides because of their overall higher antimalarial activity and the ease to synthesize a large number of derivatives. A total of 20 sulfonamide derivatives were synthesized and analysed in a reiterative process. The best activity (IC\textsubscript{50} of 10 ± 3 nM, n = 3; mean ± s.e. of the mean of n independent determinations) was obtained for compound SC09064, where a bulky 4`-sulfonamidomethyl benzene para-sulfonamide group was introduced (Fig. 1). The compound had improved physicochemical and absorption, distribution, metabolism, excretion (ADMET) properties compared with the parent molecule amicarbalide, including better solubility in water (316 versus 100 µg ml\textsuperscript{-1}), improved metabolic stability in a human microsome assay (65% residual after 1 h of incubation), negative in Ames test, and no adverse effects on HepG2 cells at a concentration of 10 µM. However, the permeability as determined in CACO 2 cells (P\textsubscript{app} = 0.28 × 10\textsuperscript{-6} cm s\textsuperscript{-1}) and the oral bioavailability (3% in rats) remained poor, which we attributed to the presence of the remaining amidine group on the West side of the molecule.

The West-side amidine group of SC09064, however, was essential for the antimalarial activity and replacing it with amines or ureatic groups rendered the compound inactive (Supplementary Table 1). We therefore explored a series of modifications of the amidine group and found that functionalizing it with a piperazine ring produced compounds with high antimalarial activity (Fig. 2; Supplementary Table 1). However, introducing the piperazine ring did not significantly improve the permeability, falling short of the targeted value of P\textsubscript{app} > 10 × 10\textsuperscript{-6} cm s\textsuperscript{-1}. The best two compounds in terms of activity and permeability were SC81458 and SC83288 with IC\textsubscript{50} values of 8 ± 1 nM (n = 6) and 3 ± 1 nM (n = 6), and P\textsubscript{app} values of 0.76 × 10\textsuperscript{-6} and 0.42 × 10\textsuperscript{-6} cm s\textsuperscript{-1}, respectively (Table 1; Fig. 3a; Supplementary Table 2). Additional criteria driving the medicinal chemistry optimization were solubility in water (Supplementary Table 2) and therapeutic efficacy in a rodent malaria model system (see below). Synthesis scheme and analytical chemistry of SC81458 and SC83288 are depicted in Supplementary Figs 1 and 2. Figure 2 summarizes the structure-activity relationship of all 172 derivatives evaluated during the course of the hit to lead optimization campaign.

SC81458 and SC83288 are fast-acting antimalarial compounds. Due to the steep dose–response curves, SC81458 and SC83288 have favourable IC\textsubscript{50} (18 and 8 nM, respectively) and IC\textsubscript{90} (50 and 20 nM, respectively) values in parasite growth assays (Fig. 3a; Table 1). SC81458 and SC83288 were also active against a range of drug-resistant P. falciparum lab strains other than Dd2, with IC\textsubscript{50} values consistently being <20 nM (Supplementary Table 3). These data suggest that SC81458 and SC83288 are able to overcome established antimalarial drug resistance mechanisms. Both compounds were also active against early stage (I–III) gametocytes, with IC\textsubscript{50} values of 76 ± 6 nM (n = 3) and...
199 ± 30 nM ($n = 3$), respectively; however, they showed negligible activity against late stage (IV and V) gametocytes (SC81458 $IC_{50} = 1.8 ± 0.2 \mu M$, $n = 3$; SC83288 $IC_{50} > 30 \mu M$). The activities against liver and insect stages were not determined.

Because of their excellent in vitro activity against $P. falciparum$ asexual blood stages, together with the opportunity to develop a needed alternative i.v. treatment for severe malaria, we further evaluate SC81458 and SC83288 despite their lack of oral bioavailability. We first investigated the stage-specific activity of the two compounds. To this end, highly synchronized cultures of Dd2-containing rings (2–4 h post invasion), trophozoites (24–26 h post invasion) and schizonts (35–37 h post invasion) were exposed to different concentrations of the compound for 6 h. Cells were subsequently washed and placed in drug-free medium until time point 10 h post invasion of the following cycle, when [3H] hypoxanthine was added for 24 h to determine cell viability. Under these conditions, both SC81458 and SC83288 exerted the highest activity against trophozoites with $IC_{50}$ values of 37 ± 3 and 40 ± 4 nM, respectively ($n = 4$; Fig. 3b,c). A slightly lower activity was observed against schizonts, with $IC_{50}$ values of 70 ± 10 and 110 ± 30 nM, respectively ($n = 4$; Fig. 3a,c). The activity against ring stages was only moderate, with $IC_{50}$ values of 5 ± 1 and 3 ± 1 $\mu M$, respectively ($n = 4$; Fig. 3b,c). Note that $IC_{50}$ values have to be interpreted in the context of the exposure time—6 h in the stage-specific assays versus 72 h in the standard proliferation assays.

To assess how quickly the two compounds were able to kill the parasite, we exposed highly synchronized trophozoites (24–30 h post invasion; 0.5% parasitemia) of Dd2 to different drug concentrations ranging from 0.1 to 10 $\mu M$ (ref. 7). Aliquots were withdrawn at different time points (from 0 to 6 h) after the addition of the drug and analysed for cell viability by [3H] hypoxanthine incorporation. Parasite clearance depended on both drug concentration and exposure time (Fig. 4a). For instance, SC83288 cleared a 0.5% parasitemia at a concentration of 500 nM within an exposure time of 3 ± 1 h ($n = 4$). Artemisinin revealed a slower killing efficacy for trophozoites under these conditions (Fig. 4a).

Since this assay underestimates parasite recrudescence, we adopted a second, standardized protocol that assessed parasite viability as a function of drug exposure time based on limiting serial dilutions of treated parasites and re-growth monitoring\textsuperscript{26}. For comparative reasons, artemisinin and atovaquone were analysed in parallel. Each drug was applied at a fixed concentration of 10 times its $IC_{50}$ value in Dd2, which were 80 and 30 nM for SC81458 and SC83288, respectively, and 19 and 10 nM for artemisinin and atovaquone, respectively. SC81458 and SC83288 cleared 99.9% of the initial parasite population (parasite clearance time (PCT\textsubscript{99.9%})) within 37 ± 4 h and 51 ± 6 h, respectively, corresponding to a parasite reduction rate of 3.4 ± 0.4 and 3.0 ± 0.5 log phases over a period of 48 h (logPRR (parasite reduction ratio); $n = 4$; Fig. 4b, Table 1). Both compounds acted quickly with a lag time of <5 h. In comparison, atovaquone is less potent as indicated by a PCT\textsubscript{99.9%} of 89 ± 7 h, a logPRR of 2.1 ± 0.4 and a lag time of >24 h ($n = 4$). Artemisinin, however, displayed a higher activity in this assay with a PCT\textsubscript{99.9%} of 21 ± 3 h, a logPRR of 4.5 ± 2 and a zero lag time ($n = 4$)\textsuperscript{26}.

**Safety profile of SC81458 and SC83288.** Profiling SC81458 and SC83288 against a panel of standardized cellular, biochemical and...
physiological assays revealed no obvious off-target activities. The two compounds did not affect \textit{in vitro} propagation of Balb C 3T3 cells, HepG2 cells, human T-cells, or human peripheral blood macrophages or showed any other signs of cytotoxicity at a fixed concentration of 50 \( \mu \text{M} \). They (10 \( \mu \text{M} \)) did not interfere with any of the 401 human kinases examined in a competition assay (Supplementary Table 4). The binding potential to 54 human transporters and receptors was low to moderate, with none of the interactions occurring in the pharmacologically relevant concentration range (Supplementary Table 5). SC81458 and SC83288 did not block human cardiac hERG-mediated \( \text{K}^+ \) conductance. hERG retained >90% of its activity at a concentration of 60 and 20 \( \mu \text{M} \) for SC81458 and SC83288, respectively. At a concentration of 100 \( \mu \text{M} \) of SC83288 the

![Figure 2](image_url)

**Figure 2 | Structure-activity relationship (SAR) analysis.** (a) Antiplasmodial activity of 172 chronologically ordered amicarbalide derivatives against the \( P. \text{falciparum} \) strain Dd2. Substitutions on the West-side amidine group (grey), the benzamidine urea linker (green) and the East-side sulfonamide group (dark blue and red) are indicated. The two compounds SC81458 and SC83288 are highlighted. (b) Summary of the SAR analysis. Colour code as above.

| Table 1 | Relevant activity parameters of SC81458 and SC83288. |
|----------|--------------------------------------------------|
|          | SC81458                                        | SC83288  |
| \( \text{IC}_{50}, \text{IC}_{90}, \text{IC}_{99} \) (nM) | 8, 18, 50 | 3, 8, 20  |
| \textit{In vitro} PCT\textit{99.9\%} (h) | 37 ± 4 (4) | 51 ± 6 (4) |
| \textit{In vitro} logPRR | 3.4 ± 0.4 (4) | 3.0 ± 0.5 (4) |
| \textit{In vitro} lag phase (h) | <5 | 5 |
| \textit{In vivo} PCT\textit{99.9\%} (%)\* | 48 | 48 |
| \textit{In vivo} logPRR\* | 5.0 (W2), 3.2 (3D7) | 3.0 |

*Obtained in the \( P. \text{falciparum} \)-infected humanized NSG mouse model system.

![Figure 3](image_url)

**Figure 3 | Susceptibility of the \( P. \text{falciparum} \) strain Dd2 to SC81458 and SC83288.** (a) Growth inhibition by SC81458 (closed circles) and SC83288 (open circles) in a standard cell proliferation assay with an exposure time of 72 h. (b,c) Growth inhibition by SC81458 (b) and SC83288 (c) against rings (R, closed circles), trophozoites (T, open circles) and schizonts (S, closed inverted triangles) after an exposure time of 6 h. The mean ± s.e.m. are shown for at least six independent replicates.
Figure 4 | In vitro killing kinetic profile of SC81458 and SC83288. (a) Concentration and exposure time-dependent clearance of a 0.5% parasitemia of trophozoites. The following drugs were investigated: artemisinin (ART, closed inverted triangles), SC81458 (closed circles) and SC83288 (open circles). (b) The graph shows the change in the number of viable parasites over time after exposure to ART (closed inverted triangles; 19 nM), atovaquone (ATO, open triangles; 10 nM), SC81458 (closed circles; 80 nM), and SC83288 (open circles; 30 nM) at a concentration corresponding to 10 times their respective IC50 values. The mean ± s.e.m. are shown for at least four independent replicates.

Figure 5 | Stability of SC81458 and SC83288 in liver microsomes. Suggested metabolic reactions and biotransformation sites of SC81458 (a) and SC83288 (b). Further detail is provided in Supplementary Figs 5 and 6.

channel remained 50% active. The inhibition potential of SC81458 and SC83288 (10 μM) towards 13 human cytochrome P450 enzymes was low and only the CYP2E1 isozyme was modestly inhibited by 30% by SC83288 (Supplementary Table 6). SC81458 and SC83288 showed no signs of genotoxicity, at 50 μM, in a reverse Ames test or of mutagenic or clastogenic activity in an in vitro micronucleus assay at 60 μM.

The maximal-tolerated i.v. bolus dose of SC83288 was at 30 mg kg⁻¹ body weight (extrapolated initial drug plasma concentration C₀ of 65,600 ng ml⁻¹) and 15 mg kg⁻¹ body weight (C₀ of 27,300 ng ml⁻¹) in rats and mice, respectively. Observed adverse effects included ataxia, respiratory symptoms and sleepiness. However, all treated animals fully recovered within 30 min after drug application. Haematological and clinical biochemical parameters assessed 24 h post application remained within normal range in all animals (Supplementary Table 7). Increasing the dose to 45 mg kg⁻¹ body weight caused apnoea in rats. The no-observed-adverse-effect level and the no-observed-effect level in rats corresponded to a tested i.v. bolus dose of 22.5 mg kg⁻¹ body weight (C₀ of 49,200 ng ml⁻¹) and 15 mg kg⁻¹ body weight (C₀ of 32,800 ng ml⁻¹), respectively.

Stability and in vitro metabolism. SC81458 and SC83288 were soluble in water (890 and 940 μg ml⁻¹, respectively) and organic solvents. Measured pK values were 5.72 and 9.78 for SC81458 and 9.82 for SC83288. The solubility in phosphate buffer decreased with increasing pH, from 1.8 mM at pH 4 to 0.4 mM at pH 9 for SC81458 and from 2.6 mM at pH 4 to 0.8 mM at pH 9 for SC83288 (Supplementary Fig. 3). The compounds were stable in different physiological solutions, including artificial gastric juice, simulated intestinal fluid, phosphate-buffered saline and human plasma, when incubated for 6 h at 37 °C.

The in vitro elimination and metabolic profile of SC81458 and SC83288 were determined using mouse, rat, dog, monkey and human liver microsomal incubations with initial compound concentrations of 10 μM. SC81458 was almost completely metabolized by monkey and human liver microsomal extracts during the 60 min incubation time, with an apparent intrinsic clearance of 303 μl min⁻¹ mg⁻¹ (Supplementary Fig. 4a). The compound was more stable in dog, mouse and rat microsomal extracts, with 37%, 54% and 74% of the initial amount remaining after 60 min of incubation, respectively (Supplementary Fig. 4a). In comparison, SC83288 was metabolically more stable. The remaining abundances of SC83288 in mouse and rat liver microsomal extracts were 96% and 91%, respectively (Supplementary Fig. 4b). In dog, monkey and human liver microsomal extracts, 83%, 51% and 50% of the compound remained after the 60 min incubation period, respectively (Supplementary Fig. 4b). In human microsomal incubations, SC83288 declined with an apparent intrinsic clearance of 75.8 ± 2.5 μl min⁻¹ mg⁻¹ (n = 3). The disappearance of SC81458 and SC83288 was mainly cofactor dependent and thus...
driven by metabolic activity in all species (Supplementary Fig. 4a,b).

The metabolite profiles of SC81458 and SC83288 were similar in all species, with minor differences in terms of the relative distribution of metabolites and the overall number of metabolites generated (Fig. 5; Supplementary Figs 5 and 6). The main biotransformations were hydroxylations (or N-oxidations), dehydrogenations, and N-dealkylations and more specifically, the cleavage of the N-butyl pipеразine chain and the cleavage of the 'East-side' 4-sulfamоxyphenyl methylene group. The resulting two major metabolites were fully characterized for their antiplasmodial activity in vitro. Metabolite 1 (M6 and M10 for SC81458 and SC83288, respectively; see Supplementary Figs 5 and 6) showed an IC_{50} value of 65 nM and metabolite 2 (M7 and M18 for SC81458 and SC83288, respectively) of 5.0 μM. Both major metabolites did not reveal off-targets effects in the standard assays described above.

SC81458 and SC83288 were stable in the presence of cultured human red blood cells and in P. falciparum cultures for at least 6 h, as indicated by the absence of metabolites or break-down products, suggesting that neither the human erythrocytes nor the parasite metabolically alter the compound (Supplementary Fig. 7).

SC81458 and SC83288 cure P. falciparum infection in mice. We next evaluated the efficacy of SC81458 and SC83288 in humanized NSG mice infected with the wild-type P. falciparum strain 3D7 or the multi-drug-resistant strain W2. At day 7 post infection when all mice had developed a patent parasitemia, mice were treated intraperitoneally (i.p.) with 0, 2.5, 5.0 and 10.0 mg kg\(^{-1}\) body weight of SC81458 or SC83288 once per day over the next 4 days. In all treatment groups, parasitemia dropped rapidly without an apparent lag phase and fell below detectable levels between day 8 and 10 post infection (with the exception of 3D7-infected mice treated with 2.5 mg kg\(^{-1}\) per day of SC81458; Fig. 6). Maximal kill rates were observed at 10.0 mg kg\(^{-1}\) per day i.p., with SC81458 and SC83288 reducing the parasite burden by ~97 and 93% within the first 24 h. Over a replicative cycle of 48 h, SC83288 reduced the parasitemia by 99.9% or three log phases (\textit{in vivo} logPRR of 3.0 and \textit{in vitro} PCT_{99.9%} of 48 h) with no distinction in activity between the two parasite strains tested (Table 1; Supplementary Fig. 8a). In comparison, SC81458 was more effective against W2 than 3D7 and the in vivo logPRR ranged from 5.3 to 3.2 for the two strains (Table 1). All mice were surveyed for possible parasitemia for the next 3 weeks. However, no recrudescence of the infection occurred, with the exception of 3D7-infected mice treated with 2.5 mg kg\(^{-1}\) of SC81458. None of the animals showed signs of discomfort or intolerability during and after the treatment regimens.

The two SC compounds were also active in the Plasmodium vinckei rodent malaria model system. SC83288 administered i.p. at a dose of 20 mg kg\(^{-1}\) once per day for four consecutive days fully cured the infection (initial parasitemia of 1.0% at time of first administration). All treated mice survived the initial infection and there was no recrudescence. In the case of SC81458, a dosing regimen of 30 mg kg\(^{-1}\) per day for 4 days reduced the parasite burden by 97% (89.9% and 3.3% parasitemia in untreated and treated mice at day 5, respectively). However, the infection persisted and all treated mice died of a recrudescent parasitemia in the days following the last administration of the compound.

Contrasting with the efficacy against P. falciparum and P. vinckei, SC81458 and SC83288 were inactive against the rodent malaria parasite Plasmodium berghei. This was the case for both an \textit{in vivo} 4 day dosing regimen of 30 mg kg\(^{-1}\) per day and an \textit{ex vivo} one-cycle growth inhibition assay. Similarly, the parental compound SC09064 (Fig. 1) revealed no activity against P. berghei and it was also inactive against P. vinckei (see Supplementary Table 8 for results on all derivatives tested in rodent malaria model systems). Differential therapeutic efficacy in various rodent model systems is a well-established phenomenon and is typically associated with charged compounds, such as positively charged amidines and negatively charged phosphoric acid or carboxylic acid containing drugs\(^{37-39}\). The phenomenon is explained based on selective uptake of charged compounds through parasite-induced channels, termed new permeation pathways, in the host cell plasma membrane and species-specific characteristics of the new permeation pathways\(^{37-39}\). Consistent with these previous findings, derivatives containing a free and hence protonated amidine group, such as SC09064, were generally inactive in the rodent malaria model systems, whereas compounds in which the amidine was functionalized as a piperazine revealed therapeutic efficacy against P. vinckei but not against P. berghei \textit{in vivo} (Supplementary Table 8).

PKs of SC81458 and SC83288. Figure 7a,b depict the PKs of SC81458 and SC83288 after a single i.p. dose in mice. In the case of SC83288, the same dosages as used in the efficacy study were administered, namely 2.5, 5.0 and 10.0 mg kg\(^{-1}\). For SC81458, a dose of 20 mg kg\(^{-1}\) was investigated. In all cases, the plasma concentrations (C_{max}) peaked within 30 min or less after drug application, suggesting a rapid entrance of the drug into the systemic circulation. The plasma concentrations then rapidly declined with an effective half time \(t_{1/2}\) of 52 min for SC81458 and 29 to 55 min for SC83288 (Table 2). The AUC_{0-\text{inf}} (area under the curve) were 4,342 and 2,019 ng ml\(^{-1}\) h\(^{-1}\) for the highest doses of SC81458 and SC83288 examined.

To better understand the PK/pharmacodynamic (PK/PD) relationship of SC83288, we analysed the \textit{in vivo} growth inhibition data (taken from Fig. 6d, time point: 2 days after commencement of treatment) as a function of the free plasma concentration, the latter being calculated from the C_{max} values by correcting for 89% plasma protein binding of SC83288 in mice (Supplementary Table 9). A sigmoidal half-maximal effective dose (ED_{50}) model was fitted to the resulting data points, yielding estimates for the ED_{50} and ED_{90} of 45 and 80 nM, respectively (Supplementary Fig. 8b). The free-minimal parasiticidal concentration (MPC) at which parasite growth was completely inhibited was 200 nM for SC83288, in good agreement with the \textit{in vitro} growth inhibition data (Supplementary Fig. 8a, see Fig. 2a for comparison).

A detailed i.v. PK of SC83288 in four different species revealed that therapeutic plasma exposures can be achieved after a bolus i.v. application (2 mg kg\(^{-1}\) body weight in mice, rats and cynomolgus monkeys and 1.7 mg kg\(^{-1}\) body weight in dogs; Fig. 7c). For instance, in cynomolgus, SC83288 reached a \(C_{0}\) of 7,338 ng ml\(^{-1}\), which corresponds to ~17 times the free MPC. The plasma protein binding of SC83288 was 82% in cynomolgus (Supplementary Table 9). The plasma level declined with a half time \(t_{1/2}\) of 16 min, a \(t_{1/2}\) of 30 min and an effective \(t_{1/2}\) of 62 min. SC83288 was cleared from the blood of the non-human primate with a rate of 470 ml h\(^{-1}\) kg\(^{-1}\). None of the animals showed any signs of intolerance during or after i.v. application of SC83288 at the concentrations indicated above. The PK properties of SC83288 were distinct from those of the parental compound SC09064 and the close amicarbalide relative imidocarb with regard to area under the curve, plasma half-life, clearance and volume distribution (Supplementary Fig. 9).

On the basis of the PK data in the four animal species, we predicted the total plasma clearance of SC83288 in humans by allometric scaling, taking into account the maximal life potential
Resistance to SC81458 and SC83288. To gain insights into the mode of action and the mechanism of resistance, we attempted to select mutant lines. No resistant parasites emerged during exposure of $10^8$ asexual blood stages to 100 nM compound for 60 days. These data suggest that the frequency of spontaneous resistance emergence is very low, possibly below the single-point mutation rate of $10^{-9}$. However, exposing a starting number of $10^{10}$ asexual blood stages to gradually increasing drug concentrations (from 50 nM to 1,000 nM) selected for resistant parasite lines during a period of ~240 days (Fig. 8a,b). Eight clonal parasite lines displaying different levels of resistance were obtained by limiting dilution and subjected to ultra-deep sequencing (Fig. 8a,b; Supplementary Table 10). All clones displayed cross resistance to both SC compounds, suggesting that SC81458 and SC83288 share a common mode of action and/or the parasites possess the same mechanism of resistance (Fig. 8c). Resistance to the SC compounds did not decrease susceptibility to licensed antimalarial drugs (Supplementary Table 11).

Of the polymorphisms and copy number variations detected in the drug selected clones three correlated with decreased susceptibility to SC81458 and SC83288 (Fig. 8d). These polymorphisms were coding mutations in Pf3D7_1447900 encoding the multi-drug resistance transporter 2 (PfMDR2; four out of eight clones with mutation), Pf3D7_0106300 encoding the Ca$^{2+}$ transporting PfATP6 (all clones had different gene modifications, six clones had mutations and four clones had duplications of the gene) and Pf3D7_1241800 encoding a putative ATP-dependent RNA helicase DBP9 (six clones; Supplementary Fig. 11).

PfMDR2 belongs to the ABC transporter superfamily and is localized at the parasite’s plasma membrane and, possibly, the digestive vacuolar membrane. PfMDR2 confers heavy metal resistance and it contributes to decreased susceptibility to atovaquone, mefloquine and quinine but not chloroquine. The non-synonymous mutation identified occurred in a repetitive region, changing a glutamic acid to aspartic acid at position 533 (Supplementary Fig. 11a). PfMDR2 is dispensable during asexual replication. The non-synonymous mutation found in several SC81458 and SC83288 selected clones results in the substitution of the amino-acid glutamic acid at position 412 in transmembrane domain 6 (Supplementary Fig. 11b). It is tempting to speculate that the introduction of the negative charge allows PfMDR2 to act on the positively charged SC compounds and expel them from the cell.
PfATP6 plays a crucial role in Ca\(^{2+}\) homeostasis in *P. falciparum* by acting as the sarco/endoplasmic reticulum (ER) Ca\(^{2+}\) pump and by facilitating Ca\(^{2+}\) transport across the parasite’s digestive vacuolar membrane. The polymorphisms identified include alanine to threonine substitutions at positions 108 and 109 within transmembrane domain 2 for SC83288 selected clones and, for SC81458 selected clones, a substitution of tyrosine for phenylalanine at position 972 within the large cytoplasmic loop (Supplementary Fig. 11c). In addition to point mutations, the PfATP6 locus and flanking regions on

![Figure 7](image-url)  
**Figure 7** | Pharmacokinetic profiles of SC81458 and SC83288. (a,b) Mean plasma concentration of SC81458 (a) and SC83288 (b) over time following i.p. administration of the indicated doses in mice. (c) Mean plasma concentration of SC83288 over time following i.v. administration of 2 mg kg\(^{-1}\) in monkeys (closed circles), mice (open circles), and rats (closed inverted triangles) and of 1.7 mg kg\(^{-1}\) in dogs (open triangles). For comparative reasons, the SC83288 AUC of the i.p. 10 mg kg\(^{-1}\) treatment group in mice is indicated in grey. The dotted lines show the lower limit of quantification (LLoQ). The mean ± s.e.m. are shown for at least three animals. Data were examined using a non-compartment analysis (NCA) and relevant PK parameters are compiled in Table 2. (d) Four species allometric scaling of the plasma clearance rate (CL) of SC83288, according to Ring et al.\(^{30}\). The CL values were corrected for the maximum life potential (MLP). The function \(y = ax^b\) was fitted to the data points, yielding values for \(a\) of 9.47 and \(b\) of 1.17 (\(R^2 = 0.997\)). Extrapolation of the fit provided estimates of the human blood clearance rate (red circle).

| Compound | SC81458 | SC83288 |
| --- | --- | --- |
| Application | i.p. | i.v. |
| Species | Mouse | Mouse | Rat | Dog | Cynomolgus |
| Dose (mg kg\(^{-1}\)) | 20 | 2 | 2 | 2 | 2 |
| AUC\(_{\text{0-inf}}\) (ng h ml\(^{-1}\)) | 4,342 | 646 | 891 | 1,419 | 4,375 | 2,262 | 4,253 |
| \(C_0\) (ng ml\(^{-1}\)) | 0 | 0 | 3,644 | 4,375 | 5,579 | 4,564 | 6,569 |
| \(C_{\text{max}}\) (ng ml\(^{-1}\)) | 7,384 | 610 | 2,560 | 3,283 | 4,564 | 6,569 |
| \(t_{\text{max}}\) (min) | 30 | 5 | 5 | 5 | 5 |
| \(t_{\frac{1}{2} \text{ initial}}\) (min) | NC | NC | 9 | 9 | 12 | 16 |
| \(t_{\frac{1}{2} \text{ terminal}}\) (min) | 860 | 100 | 43 | 625 | 90 | 580 | 320 |
| \(t_{\frac{1}{2} \text{ effective}}\) (min) | 52 | 33 | 12 | 52 | 35 | 62 |
| MRT\(_{\text{0-t}}\) (h) | 1.24 | 0.79 | 0.28 | 1.25 | 0.84 | 1.50 |
| CL (ml h\(^{-1}\) kg\(^{-1}\)) | 4,607 | 3,870 | 2245 | 1,410 | 884 | 470 |
| \(V_c\) (ml kg\(^{-1}\)) | NC | NC | 549 | 457 | 359 | 273 |
| \(F\) (%) | 62 | 58 | 100 | 100 | 100 | 100 |

AUC, area under the concentration-time curve; \(C_0\), initial drug plasma concentration; \(C_{\text{max}}\), observed maximum plasma concentration after administration; CL, total plasma clearance of drug after administration; \(F\), relative bioavailability compared to i.v.; MRT, mean residence time of the unchanged drug in the systemic circulation; NC, not calculated; \(t_{\text{max}}\), time of \(C_{\text{max}}\); \(t_{\frac{1}{2}}\), time required for the concentration to fall to 50% of its initial value; \(V_c\), apparent volume of the central or plasma compartment.

Data were examined using a non-compartment analysis.
chromosome 1 were duplicated in SC83288 selected clones (Fig. 8d). The duplications were of different nature. One clone had the complete chromosome 1 duplicated, two clones shared the same domain duplicated, but all four clones duplicated a total of six genes, including PfATP6 (Supplementary Fig. 11d).

To understand if any of the mutations identified in the three candidate genes might exist already in the *P. falciparum* population, we checked the 3,500 samples from the MalariaGen consortium (https://www.malariagen.net/data/catalogue-genetic-variation-p-falciparum-v4.0). However, none of the mutations were found; all were de novo.

The presence of both point mutations and gene amplification is striking and suggests a critical role of PfATP6 in SC83288 resistance and, possibly, the antiplasmodial mode of action. If SC83288 and SC81458 target PfATP6 then inhibition of this pump would incapacitate the ER of its function as a Ca$^{2+}$ store and the concentration of free Ca$^{2+}$ would increase in the parasite’s cytoplasm. To investigate this hypothesis, we loaded *P. falciparum*-infected erythrocytes with the Ca$^{2+}$ sensitive fluorochrome Fluo-4 and recorded dynamic changes in cytoplasmic free Ca$^{2+}$ in a live cell confocal set-up. However, treatment of the cells with SC83288 (10 and 20 μM) or SC81458 (10 and 20 μM) did not affect cytoplasmic free Ca$^{2+}$ (Supplementary Fig. 12). In comparison, cyclopiazonic acid (CPA; 10 μM), an established inhibitor of PfATP6 (ref. 37), induced a strong Ca$^{2+}$ responses in the parasite’s cytoplasm (Supplementary Fig. 12). These findings suggest that PfATP6 plays a role in resistance to the SC compounds, but it does not seem to be the molecular target.

**Discussion**

SC81458 and SC83288 fulfil several criteria of the target candidate profile for a molecule against severe malaria. Both compounds...
have advantageous parasitological properties. They display steep, in vitro grow inhibition curves with IC_{90} values < 10 nM and IC_{99} values ≤ 50 nM (Table 1) and absolute Hill coefficients larger than unity (2.6 ± 0.3 for both compounds, Fig. 2a). We interpret this concentration–response behaviour as evidence of a biological threshold phenomenon. SC81458 and SC83288 act quickly, killing > 99.9% of the parasites within the first 48 h of treatment as demonstrated in a standardized in vitro assay,36,38 and in the humanized NSG mouse model system (Figs 4 and 6). The parasite reduction rate of three log units is a conservative estimate given that, in human patients, parasite killing is aided by the host’s immune system and splenic entrapment and clearance mechanisms.29,30 A logPRR of ≥ 3.0 is considered a favourable property of an antimalarial drug candidate, although the value is lower than that of artemisinin.31 While the compounds are primarily active against replicative blood stages, with trophozoites being the most susceptible stage, they also target early stage (I–III) gametocytes. Being devoid of activity against the late stage gametocytes (IV and V) the compounds may not exert appreciable transmission-blocking activity. In vitro pharmacokinetic profiling discovered no obvious liabilities. Moreover, all animals tolerated exposure to the compounds at therapeutic concentrations without any signs of distress or discomfort. However, a full evaluation of the safety of the compounds awaits further toxicological studies, in particular acute and 28 day repeat-dose toxicology in a non-rodent species.

Both SC81458 and SC83288 belong to a chemotype that has not yet been used in malaria chemotherapy and that does not reveal cross resistance to currently used antimalarials. The analysis of SC83288- and SC81458-resistant clones provided first clues about the possible resistance mechanisms and/or the mode of action. Among the three candidate genes associated with resistance PfED7_0106300 encoding the Ca^{2+}-transporting PfATP6 stands out. The finding of both point mutations and gene duplications suggests that PfED7_0106300 is under strong selective pressure by the SC compounds. PfATP6 is the sarco/ER Ca^{2+} pump of the parasite, and as such plays a vital role in Ca^{2+} homoeostasis. PfATP6 was once considered a putative target of artemisinin.39,40 However, more recent studies failed to validate this hypothesis and, instead, point towards a mode of action of artemisinin that involves inhibition of a phosphatidylinositol-3-kinase.41

SC81458 and SC83288 do not seem to target PfATP6. At least no Ca^{2+} responses were induced in the parasite’s cytoplasm on the addition of the SC compounds, as would have been expected if SC81458 and SC83288 were to directly inhibit PfATP6. This suggests a putative role of PfATP6 in the drug resistance mechanism, possibly by acting as a compensatory mechanism for the yet to be identified molecular target of the SC compounds. Alternatively, mutated and amplified PfATP6 might affect the intracellular distribution of the compounds by transporting the compounds into a compartment, where the compounds are less harmful to the parasite.

A comparable role in resistance can be envisaged for PfMDR2, which when mutated might expel the SC compounds from the cell. A function of PfMDR2 as an export system is consistent with the subcellular localization of PfMDR2 at the parasite’s plasma membrane and the fact that PfMDR2 confers heavy metal tolerance and contributes to decreased susceptibility to several antimalarial drugs including quinine and atovaquone.35,36

The role of the putative ATP-dependent RNA helicase DBP9 in the mode of action/mechanism of resistance of the SC compounds is less clear (Fig. 8). RNA helicas are not yet considered drug targets in malaria research, although specific inhibitors have been developed in other systems.

Although both SC compounds are able to cure a P. falciparum infection in humanized NSG mice, SC83288 showed improved metabolic stability, better efficacy over a wider dose range and less strain variability, compared with SC81458 (Fig. 6; Table 1)—therefore nominating SC83288 as a potential preclinical development candidate. The dose finding experiments conducted in the mouse challenge model, together with the resulting PK/PD relationship, provide first estimates of dose projections of SC83288 in humans. Taking into consideration that the initial treatment against severe malaria is i.v. applied until the patient is able to take oral medication for final parasite elimination, the mouse PK/PD data suggest that a steady-state free plasma concentration of 1.5 × the free MPC of 200 nM maintained over a period of < 8 h suffices to reduce the initial parasite burden by 99.9% within 48 h (Supplementary Fig. 8a). Assuming a parasitemia of 10% that is not uncommon in patients with severe malaria, the total parasite burden will fall from its initial value of ~ 10^{12} infected erythrocytes (assuming a total red blood cell count of 2 – 3 × 10^{12}) to ~ 10^9 or below if the additional contribution of splenic clearance and immune-killing mechanisms are considered. The parasite burden may even fall faster with longer exposure times, as suggested by the in vitro killing speed experiments revealing concentration and exposure-dependent parasite killing kinetics (Fig. 4b). A free plasma concentration of 1.5 × the free MPC of 200 nM translates to a total plasma concentration of 860 ng ml^{-1} (assuming 78% plasma protein binding, Supplementary Table 9). Maintaining such a plasma concentration would require infusion rates between 8.8 and 13.1 mg h^{-1} given a total plasma clearance rate between 1.0250 ml h^{-1} (lower estimate) and 1.150 ml h^{-1} (higher estimate; Fig. 7d). How long such an infusion should be administered or whether i.v. or intramuscular bolus applications suffice would await efficacy studies in clinical settings. The projected dosing regimen would maintain a safety margin of ~ 55-fold assuming comparable i.v. no-observed-adverse-effect level in rats and humans with a C_{90} of 49,200 ng ml^{-1}. In summary, SC83288 may not match the favourable pharmacological and PDs properties of artemisinin in all aspects. However, the attributes of SC83288 have to be considered in light of the emerging resistance against artemisinin derivatives and the adverse side effects observed in a substantial number of patients treated with parenteral artemesetum.16,17 The role of SC83288 as an alternative chemotherapeutic option will require extensive clinical studies. However, the available preclinical data suggest that SC83288 holds promise for an efficacious and safe clinical candidate for the acute treatment of severe malaria.

Methods

Ethical clearance. All animal studies were carried out in strict accordance with national and European guides for the care and use of laboratory animals (European regulations; 2010/63/EU). The challenge studies using humanized NSG mice were approved by the Centre d’Expérimentation Fonctionnelle (CEF, La Pitie-Salpetrière, Paris) and the Ministère de l’Education Nationale, de l’Enseignement Supérieur et de la Recherche (authorization number 01737.03). All procedures pertaining to the PK studies were in compliance with the German Animal Welfare Act and German regulations (TierSchG/TierSchVersV) and were approved by the Regierung von Oberbayern, München, (authorization number 55.2-1.4-2532.2-9/11; studies in mice and rats) and the Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit Oldenburg (authorization numbers: 33.2-42502-05-LG-01/86/2012 and 33.2-42502-05-LG-01/2014/04 SA; studies in cynomolgus monkeys and dogs).

Chemicals. The following chemicals were purchased from the following vendors: Sigma-Aldrich: sodium/potassium ATPase inhibitor ouabain, artemisinin, atovaquone, 4-(chlorosulphonyl)phenyl isocyanate, 4-nitro-sulphonyl-4-aniline and acetotriol. Fisher Scientific: isopropanol, dimethylacetamide, dichloromethane, dimethyformamide, diisopropylethamine, 3-nitrobenzamidine, 3-amino-benzenemidazin; tin(II) dichloride dihydrate, formic acid, acetotriol, 4-trifluoromethylalanine, 3-chloroalanine, 2-bromoalanine, 2-amino-benzenonitrile.
2-bromo-6,6-difluoroaniline, 3-tri-fluoromethyl-4-chloraniline, 3-trifluoromethyraniline, 2-bromo-4-trifluoromethyraniline, 3,6-bis-trifluoro-
methyraniline, 2,6-difluoro-4-amidinomethyl-aniline, sodium fluoride, oxalic acid and ammonium acetate. Acros Organics: 4-aminosulfonamide, 
3-isocyanatobenzonitrile, n-butylpiperazine, 4-hydroxypiperidine, piperazine, silica gel, 1H- (4-4-(bromophenyl)isonitril), 4-(4-nitrophenyl)isocyanitil), 4- (4-chlorophenyl)isonitril) and methyl sulfoxide-d6. Maybridge Chemical Comp,
SC81458 (4-(3-(3-(4-sulfamoylbenzyl)sulfamoyl)phenyl)ureido) phenyl)methyl)carbonyl-1-carboxylate) was prepared from 4-(3-
(3-cyanophenyl)ureido)-N-(4-sulfamoylbenzyl)sulfenamidone using 1 equivalent of methyl piperazine-1-carboxylate as a secondary amine. The product was purified by preparative HPLC. Yield: 21%; [M + H] + calculated for C23H21N5O6S2, 486.090; found for [M + H] + : 486.087. 1H-NMR (75 MHz, DMSO-
45.55, 111.64, 118.00 (2 $\times$ C), 132.42 (2 $\times$ C), 141.24, 140.19, 141.98, 142.91, 143.08, 152.16. Steps (ii and iii): production of the cyanourea compound (4). An amount of 
[(3-(4-sulfamoylbenzyl)sulfamoyl)phenyl)methyl) piperazine-1-carboxylate) was prepared from 4-(3-(3-cyanophenyl)ureido)-N-(4-sulfamoylbenzyl)benzenesulfonamide using 1 equivalent of methyl piperazine-1-carboxylate as a secondary amine. The product was purified by preparative HPLC. Yield: 21%; [M + H] + calculated for C35H31N5O6S2, 628.1; 1H-NMR (300 MHz, DMSO-
5% O2, 92% N2, and at a humidity of 99%. Cells were grown at a haematocrit of 5.0% and at a parasitemia of no > 5%.

**IC50 measurement.** Growth inhibition assays were performed according to standard protocols based on the detection of parasitic DNA by fluorescent SYBR green staining or by [3H] hypoxanthine incorporation22. Briefly, a previously sorbitol-synchronized culture of P. falciparum Dd2 ring-stage parasites was incubated in the presence of decreasing drug concentrations in a 96-well black microtiter plate, at the following final conditions: 100 μl per well, 0.5% parasitemia, 2% haematocrit, incubated for 72 h at 37 °C. 1 equivalence of drug was added to two different protocols: first, we adapted the previously established protocol by King et al.44. Briefly, blood cultures were grown in 10 ml petri dishes at 37 °C under controlled atmospheric conditions of 3% CO2, 5% O2, 92% N2 and a humidity of 99%. Cells were grown at a haematocrit of 5.0% and at a parasitemia of no > 5%.

**In vitro speed of action.** In the in vitro speed of action of SC81458, SC83288, artemisinin and artemovase on Dd2 P. falciparum parasites was assessed as described in the following sections. Growth inhibition assays were performed according to two different protocols: first, we adapted the previously established protocol by Sand et al.25 and performed the assay using standardized conditions. Briefly, asynchronous cultures (0.5% parasitemia and 2% haematocrit) with a predetermined ring population were treated with the selected drugs at concentrations corresponding to 10 times their respective IC50 values. Drugs were renewed daily over the entire treatment period. Samples of untreated (0 h) and treated parasites (8, 24, 48, 72, 96, 120, 144 and 168 h) were aliquoted for perform liquid chromatography using a gradient of MeOH in DCM mixtures as eluent. [M + H] + was measured in positive ion mode. The samples were injected on a Waters 2,700 Autosampler, Waters 1,525 and SC83288 (see below and Supplementary Note 1). Analytical HPLC–MS/C2H9 were performed with Waters 2,700 Autosampler, Waters 600 Multisolvent Delivery System with preparative pump heads (500 μl), Waters 600S Controller and Waters ZQ single quadrupole mass spectrometer with electrospray source. Column: Chromolith Fast Gradient C18 (Merk). 50 × 2 mm, with stainless steel 2 mm, with stainless steel 2 mm, with stainless steel 2 mm, with stainless steel 2 mm, with stainless steel 2 mm, with stainless steel 2 mm. Eluent A, H2O; B, MeCN. Preparative HPLC–MS were performed with a Waters 1,525 pump heads (500 μl), Waters 600S Controller and Waters ZQ single quadrupole mass spectrometer with electrospray source. Column: Waters X-Terra RP18, 5 μm, 19 × 150 mm. Eluent A, H2O + 0.1% HCOOH; eluent B, MeCN. Different linear gradients were used, as usually adapted to sample. Analytical HPLC–MS for PK analysis were performed with Agilent 1100 HPLC System, HYS-Pal Autosampler and Micromass Ultima triple quadruple, mass spectrometer with electrospray source. Column: Chromolith RP18, 50 × 4.6 mm. Eluent A, H2O + 0.1% HCOOH; eluent B, MeCN. H2-NMR and 13C-NMR were performed with a Bruker AV300 (300.13 MHz) at a temperature of 305 K. The compounds described in this document were prepared according to methods described in the following synthesis method. Steps (i and ii): production of the cyanourea compound (4). An amount of 28 mmol of 3-amino benzimidazole (1) was dissolved in 40 ml dichloromethane (DCM). An amount of 27 mmol of 4-isocyanatobenzene-1-sulfonyl chloride (2) was added portionwise. The reaction was allowed to 

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56.78, 116.99, 117.57 (2 $\times$), 120.75, 121.18, 125.55 (2 $\times$), 127.66 (2 $\times$), 129.81 (2 $\times$), 129.85, 132.42, 141.09, 142.02, 142.91, 144.17, 152.94, 163.94, 167.24 (HCOO -). element. anal.: element: (calc. comp. incl. add. (%)/found (%)): C: (53.56/51.82), H: (5.69/5.9), N: (14.57/14.16), S: (9.53/9.2). Oxygen not determined. 13C-NMR (75 MHz, DMSO-d6) δ = 13.81, 19.95, 28.23, 45.56 (3 $\times$), 51.76 (2 $\times$), 78.3-78.36 (m, 1H), 7.99-8.12 (m, 1H), 8.53 (s, 1H), 11.19-11.29 (m, 2H).
In vitro metabolism in liver microsomes. The study was conducted by Novamass Ltd (Oulu, Finland). The in vitro disappearance and metabolite profile of SC14158 and SC38288 was determined using mouse, rat, dog, monkey and human liver microsomes isolated from fresh (in vitro) or frozen (in vivo) liver samples. In vitro metabolism was conducted using pooled human liver microsomes (0.5 mg of microsomal protein per milliliter) in the presence of the appropriate cofactor. Two parallel incubates, one with cofactor and one without, were employed. Each reaction mixture was preincubated for 2 min at 37 °C. Reaction was started by addition of 1 mM NADPH and 1 mM UDPGA. After incubation period of 0 or 60 min, samples were collected and the reaction was terminated by addition of ice-cold acetonitrile. Samples were subsequently cooled in an ice bath and centrifuged (10 min, 16,100 × g). The supernatants were transferred to maximum recovery vials and analysed by LC/MSMS.

Determination of efficacy in the P/R/NSG mouse model. NSG male mice aged 9–11 weeks (Charles River, US) were used for this study. They were kept in sterile isolators, provided with UV light-exposed commercial food and autoclaved water ad libitum. Human red blood cells (HRBC) were obtained from the Etablissement Français du Sang (Ile-de-France, Rungis). HRBC were washed twice with RPMI-1640 medium (Gibco/BRL) by centrifugation at 900g, 10 min at 25 °C resuspended in RPMI-1640 medium and kept at 4 °C for a maximum of 2 weeks. Mice humanization and P. falciparum infection was carried out as previously described47. Mouse tissue macrophages were depleted by clodronate, provided by Roche Diagnostics (Mannheim, Germany), encapsulated in liposomes (lip-clod) as previously described48. Neutrophils were controlled using the monoclonal antibody NIMP-R14 (the monoclonal antibody NIMP-R14 was purified from a hybridoma provided by Dr M. Strath from the National Institute for Medical Research, London, UK)49. On day 0, each mouse was injected i.p. with a dose of 10 mg kg⁻¹ of mAb NIMP-R14, mixed with 60 μl of lip-clod (300 μg of clodronate). On days 2 and 4 each mouse received, i.p., 0.5 ml of HRBC mixed with a dose of 10 mg kg⁻¹ of mAb NIMP-R14 and 60 μl of lip-clod. On day 8, each mouse was injected with 0.5 ml of HRBC infected with P. falciparum strains at a parasitemia of 0.3%, mixed with a dose of 10 mg kg⁻¹ of mAb NIMP-R14 and 60 μl of lip-clod. Following the P. falciparum infection, a dose of 10 mg kg⁻¹ of mAb NIMP-R14 and 60 μl of lip-clod was injected every 2–3 days. The haematocrit and gill haematocrit in the peripheral blood of mice was followed up during the assay in blood samples taken from the tail. After the P. falciparum infection, the HRBC graft was carried out every 2–3 days except in those mice where their haematocrit was up to 60% and the percentage of HRBC higher than 60%. Those mice only received the immunosuppressor treatment and the graft of HRBC was carried out again once the haematocrit decreased to 50%.

PK studies. On the treatment day, all animals were weighed and the dosing volume was calculated for each individual animal according to its actual body weight. All animals were dosed once. Blood samples were taken at different time points and plasma was collected after centrifugation (10,000g, 10 min, 4 °C). Plasma samples were analysed by LC-MS/MS. During and after the application, animals were observed for clinical signs and mortality. All relevant observations were recorded. The PK studies in cynomolgus monkeys and beagle dogs were performed by the Laboratory of Pharmacology and Toxicology (LPT), Hamburg, and PK and toxicity studies in mice and rats were performed by the 4SC Discovery GmbH, Munich. PK parameters were obtained by a non-compartment analysis, using Kinetica 4.1 (Thermo Scientific).

In vitro generation of drug-resistant P. falciparum lines. To identify the mode of action/mechanism of resistance of SC14158 and SC38288, a chemogenomic approach was used, whereby resistant parasites were obtained by in vitro drug pressure selection and subjected to whole-genome sequencing to identify the genetic basis of resistance. Three independent selections of Dd2 parasites at an initial population of 10⁶ infected red blood cells (performed in 150 ml flasks, 2% haematocrit) were subjected to SC14158 or SC38288 at a starting concentration of 10 μM. The drug pressure was gradually increased by increments of 50 nM up to 1 μM according to the parasite fitness. Drug resistance was confirmed by measuring the IC₅₀, after two consecutive cycles in the absence of drug, using a SYBRGreen growth inhibition assay as previously described50. After up to 200 days of drug pressure, resistant drug-resistant lines were cloned and dilution for single-cell isolation and genomic DNA was extracted using the DNeasy Blood&Tissue kit (Qiagen, USA) and subjected to ultra-deep sequencing.

Whole-genome sequencing of isolates. Genomic DNA was extracted from eight clones selected for reduced susceptibility to SC38288 and SC14158 resistant clones and 100 Dd2 parent, Illumina paired-end sequencing was generated using a PCR-free protocol51 and sequenced using an Illumina MiSeq obtaining 150 bp reads. The sequence data have been deposited in the European Nucleotide Archive (ENA) under the accession code ERP005793 (https://www.ebi.ac.uk/ena/data/search/query?ERP005793). The accession numbers of the reads are compiled in Supplementary Table 10.

Bioinformatics analysis. The analysis was performed as described3,14. In short, to improve mapability the P. falciparum reference genome 3D7 was transferred into the Dd2 parent clone by repeatedly mapping the reads and correcting the differences using iCORN2 (ref. 51). New reads from this next generation was annotated with RATT51. Reads from all samples (Supplementary Table 10) were mapped against the Pfam reference genome using bowtie2 (ref. 53). Variants were called using two methods: (i) the genome analysis toolkit (GATK) single-nucleotide polymorphism calling pipeline52, using the settings for Plasmidium; and (ii) mpileup and varfilter (varFilter -D 2000; Quality ≥ 60) from the SAMTools package53. A bespoke Perl script returns genes where just drug-resistant sample had single-nucleotide polymorphisms. Last, insertions, deletions, insertions and duplications were manually detected using BAMVie56.

Ca²⁺ live imaging. Trophozoite-stage P. falciparum HB3 parasites were incubated with 10 μM of Flu-4-AM in RPMI 1640 medium (Life Technologies) with Pluoronic F-127 (0.1% (v/v)) and 40 μM of probenecid for 40 min at 37 °C, as previously described52. Dye-loaded parasites were washed twice before settling onto poly-L-lysine-coated glass slides. Confocal laser scanning fluorescence microscopy (CSFM) was performed using a Leica TCS SPS (Leica Microsystems CMS GmbH). Flu-4-AM was excited at 488 nm (argon laser, 0.03%) and the emitted fluorescence was collected from 505–520 nm. Single images were obtained using a 63 × objective, with a fivefold software zoom, 1,024 × 1,024 pixels, every 2 s over a time span of 120 s. The Ca²⁺ ATPase inhibitor CPA, the Na⁺/K⁺ ATPase inhibitor ouabain, SC14158 and SC83288 were added to a final concentration of 10 μM at 45 °C. The calcium signal was further analysed using Fiji, and the mean fluorescence signals were compared using one-way analysis of variance in SigmaPlot 13 (Systat Software Inc.).

Gametocytocidal activity. Activity against early- and late-stage gametocytes was determined in miniaturized 384-well plates assays as previously described59,60. Briefly, gametocytes from a recombinant line Nf5450,61, expressing a green fluorescent protein–luciferase reporter gene under the gametocyte-specific Pfas16 promoter were induced and purified as previously described59; gametocytes were then treated either on day 1 (stage 1 gametocytes) or day 8 of gametocytes (stage IV gametocytes) with serially diluted SC14158 or SC38288 to achieve a final top concentration of 40 μM (0.4% dimethylsulfoxide (DMSO)). After 72 h of incubation, gametocyte viability was assessed by luminometry, after addition of SteadyLight plus luciferase detection kit (PerkinElmer) to the plates58,59.

Plasma protein binding of SC38288. The study was carried out by Novamass Ltd (Oulu, Finland). The plasma protein binding of SC38288 was determined in human rat, mouse and cynomolgus plasma, using a method based on rapid equilibrium dialysis and LC/MS/MS analysis. Briefly, plasma from the different species was incubated with a single concentration of SC38288 for 10 min at 4 °C at 37 °C in a rapid equilibrium dialysis device. Proteins were then precipitated by acetonitrile addition containing a phenacitin internal standard. After centrifugation (10,000g) the supernatants were analysed by LC/MS/MS to obtain the protein unbound fraction of the analyte substance.

Inhibition of cytochrome P450 (CYP) enzymes. The study was conducted by Novamass Ltd (Oulu, Finland) according to a previously established protocol62. The in vivo potentiation of SC14158 and SC38288 toward the CYP enzymes was investigated using cytochrome P450 (CYP) specific substrates and 13 probe reactions for nine major drug-metabolizing CYP enzymes in incubation with a pool of human liver microsomes. Briefly, each incubation mixture contained 0.5 mg microsomal protein per ml, 0.1 M phosphate buffer (pH 7.4) and the 10 probe substrates, at the following concentrations: melatonin (CYP1A2, 5 μM), coumarin (CYP2A6, 2 μM), bupropion (CYP2B6, 2 μM), amodiaquine (CYP2C8, 5 μM), tolbutamide (CYP2C9, 8 μM), omeprazole (CYP2C19 and CYP3A4, 5 μM), dextromethorphan (CYP2D6, 1 μM), chloroxazone (CYP2E1, 10 μM), midazolam (CYP3A4, 1 μM) and testosterone (CYP3A4, 30 μM). SC14158 and SC38288 were dissolved into DMSO and added into the incubation mixture to a final concentration of 10 μM. The reaction mixture was preincubated for 2 min at 37 °C before the reaction was initiated by addition of 1 mM NADPH. Each reaction was terminated after 20 min by addition of ice-cold acetonitrile containing a phenacitin internal standard. The samples were subsequently diluted in an ice bath to precipitate the proteins centrifuged (10 min, 16,200 × g). The supernatants were transferred to a Waters Max Recovery vial and analysed by LC/MSMS.
Screening for kinase inhibition of SC81458

The KINOMEScan was carried out by Ambit (San Diego, USA) according to a previously established protocol. The experimental competition binding assays measure the ability of a compound to compete with an immobilized, active-site directed ligand. The assay is performed by combining three components: DNA-tagged kinase; immobilized ligand; and a test compound. The ability of the test compound to compete with the immobilized ligand is measured via quantitative PCR of the DNA tags.

For most assays, kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an Escherichia coli host derived from the BL21 strain. E. coli were grown to log-phase and infected with T7 phage from a single stock and incubated with shaking at 32 °C until lysis (90–130 min). The lysates were centrifuged (6,000g) and filtered (0.2 μm) to remove cell debris. The remaining kinases were produced in HEK-293 cells (Sigma Aldrich, catalogue number: 85120602) and subsequently tagged with DNA for quantitative PCR detection. Strepavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 min at RT to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (1% BSA, 0.05% Tween 20, and 0.5 mM dithiothreitol). SC81458 was prepared as 40x stock in 100% DMSO and directly diluted into the assay to a final concentration of 10 μM. All reactions were performed in polypropylene 384-well plates in a final volume of 0.04 ml. The assay plates were incubated at RT with 0.05% Tween 20 and 0.5 mM sodium deoxycholate to remove unbound ligand and to reduce non-specific phase binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and SC81458 in 1 x binding buffer (20% Seaplaque, 0.17 x PBS, 0.05% Tween 20, 6 mM sodium deoxycholate). SC81458 was then resuspended in elution buffer (1% BSA, 0.05% Tween 20, and 0.5 mM sodium deoxycholate) and incubated at RT with shaking for 30 min. The kinase concentration in the eluates was measured by quantitative PCR.

In vitro safety pharmacology profiling. The study was carried out by Cerep (Celle l’Evescaut, France). An in vitro pharmacology profiling panel was designed to detect potential high-risk clinical adverse drug reactions. The panel comprised 54 targets. For assessment of activity against these targets, SC81458 and SC83288 were tested in an eight-point concentration-response covering up to a test concentration of 10 μM and an IC50 and Ki were determined using radioligand binding assay. The specific ligand binding to the receptors was defined as the difference between the total binding and the non-specific binding determined in the presence of an excess of unlabelled ligand. Results were expressed as a per cent of control from concentration–response curves. The IC50 values were determined by non-linear regression analysis of the competition curves generated with mean residual sum of squares, Akaike information criterion and coefficients of variation between the total binding and the non-specific binding determined in the presence of the test compounds. Results showing an inhibition (or stimulation) due to the different in vitro metabolite profile (Supplementary Fig. 5b). Parameters for the other species could be derived with acceptable precision (Supplementary Fig. 10b).

The mPBPK model was used to simulate the human PK and various dosing schedules to obtain anticipated pharmacological active exposures, using the i.v. route and different infusion protocols. The rate of infusion C0 was defined by the equation C0 = f0 × CL, where f0 is the steady state blood level and CL is the total plasma clearance rate. The simulations were based on point estimates of the relevant parameter. Variability was not accounted for. Model fitting and simulations were performed using PhoeniX4/WinnNonLin 6.4 on an Intel Core i5 processor.

Data availability. The authors declare that the data supporting the findings of this study are available in the article and its supplementary information files, or available from the authors upon request. The whole genome sequences of the P. falciparum clone D2 and 8 P. falciparum clones resistant to SC83288 and SC81458 have been deposited in the European Nucleotide Archive (ENA) under the accession code ERP005793 (https://www.ebi.ac.uk/ena/data/search?query=ERP005793).

Alometric scaling and prediction of human PK. An empirical four species simple allometry according to the rule of exponents was used in predicting the human total plasma clearance of SC83288 (ref. 30). The average body weights were as follows: mouse, 0.0287 kg; rat, 0.267 kg; monkey, 4.17 kg; and dog, 9.9 kg. Values for the maximum lifespan potential were taken from Boexenbaum (1982) and are: mouse, 2.66 years; rat, 4.48 years; monkey, 22.46 years; and dog, 22.20 years. For man, a body weight of 70 kg and a maximum lifespan potential of 92.22 years were assumed. For a simple allometry with plasma protein binding correction only mouse, rat and monkey were used since data on plasma protein binding of SC83288 were only available for these three species. The preclinical plasma concentration versus time profiles were analysed using a mPBPK model. A two tissue compartment version of the mPBPK model provided the best fits. Total plasma clearance was parameterized as CL = rTBW (TBW, total body weight). Parameters a and b were derived from the mPBPK model. Volumes of distribution were implemented as V1 + V2 + Vp = TBW, where Vp equals the plasma volume of each species and V1 was parameterized as V1 = rTBW. Fractions of cardiac output f1d and f2d satisfied the condition f1d + f2d ≤ 1. Values for cardiac output were taken from Gabrielson and Weiner. A hematocrit of 0.42 was assumed for conversion to plasma flows. The parameters a, b, c, f1d, f2d and Ks (tissue/plasma partition coefficient) were estimated from a global fit of all data, assuming a common f1d, f2d and Ks for all species. Data below the lower limit of quantification were excluded from the fit. A 1/ypest weighting scheme was applied. Assessment of model quality included visual inspection of the fitted curves, residuals sum of squares, Akaike information criterion and coefficients of variation of the estimated parameters. In the final model dog data were excluded since the data could not be fitted together with the other three species, which was possibly due to the different in vitro metabolite profile (Supplementary Fig. 5b). Parameters for other species could be derived with acceptable precision (Supplementary Fig. 10b).

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Stability in cultured blood cell cultures. An enriched young trophozoite-stage culture of P. falciparum D2 was obtained by magnetic purification and allowed to recover for 1 h at 37 °C in RPMI 1640 medium (Life Technologies) enriched with 1% hypoxanthine (C.C.Pro GmbH) and 10% Albumax (Life Technologies). The experiment was performed in a total volume of 300 μl at a haematocrit of 50%, in presence or absence of 2 μM of SC81458 or SC83288, for 6 h at 37 °C under 5% CO2. Cells were routinely split every 3–4 days at a 1:5–1:10 ratio. The electrophysiology of the measurements were performed using the whole cell patch-clamp method. Extracellular bath solution contained 137 mM NaCl, 4 mM KCl, 1 mM MgCl2, 1.8 mM CaCl2, 10 mM glucose, 10 mM HEPES (pH 7.4 with NaOH), the intracellular pipette solution 130 mM KCl, 1 mM MgCl2, 5 mM MgATP, 5 mM EGTA and 10 mM HEPES (pH 7.2 with KOH). To assess the effects of SC81458 on the current the SC81458 was added to 10 μM to the HEPES containing 24 mM of conditioning voltage pulse was applied every 20 s by the holding membrane potential (–80 mV) is carried out to +40 mV followed by a 500 ms test pulse of +40 mV to –40 mV. The amplitude of the current peak induced by the test pulse was determined at equilibrium (Ieq) and after (Ieq) addition of the substance. After this, the current was recovered to the baseline and both solutions until a complete recovery of the current occurred; otherwise, the cells were replaced with new ones.
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Erratum: SC83288 is a clinical development candidate for the treatment of severe malaria

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This Article contains errors in Figs 1 and 8 that were introduced during the production process. The compound on the lower right side of Fig. 1 is labelled incorrectly and should be labelled ‘SC83288’. The correct version of Fig. 1 appears below as Fig. 1. In Fig. 8c, ‘SC83458 selected’ should read ‘SC81458 selected’ and in Fig. 8d ‘SC81758’ should read ‘SC81458’. The correct version of Fig. 8 appears below as Fig. 2.

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**Figure 1**

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**Figure**
Figure 2