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Identification and Optimization of an Aminoalcohol-Carbazole Series with Antimalarial Properties

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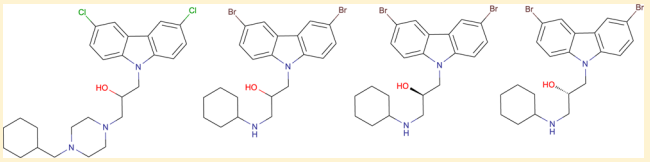
Supporting Information

ABSTRACT: Recent observations on the emergence of artemisinin resistant parasites have highlighted the need for new antimalarial treatments. An HTS campaign led to the identification of the 1-(1-aminopropan-2-ol)carbazole analogues as potent hits against *Plasmodium falciparum* K1 strain. The SAR study and optimization of early ADME and physicochemical properties direct us to the selection of a late lead compound that shows good efficacy when orally administrated in the in vivo *P. berghei* mouse model.



KEYWORDS: *Malaria, WHO, SAR, carbazole, Plasmodium falciparum, Plasmodium berghei, IC_{50}, hERG*

One of the most mortal parasitic diseases remains malaria, with an estimated one million deaths per year.[1] Even if *Plasmodium* species, the parasite responsible for malaria, often infects humans, the *Plasmodium falciparum* strains are responsible for most of the death caused by the disease.[2] The main affected populations are children under five years old and pregnant women.[3] Dramatically one child dies from malaria every 30 s.[1,4]

The extensive use of well-known antimalarial drugs led to the emergence of resistance of certain strains to the current marketed drugs (e.g., chloroquine, Figure 1)[5] urging the international community to develop new chemotherapeutic agents. The discovery of a highly active compound (artemisinin, Figure 1)[6] led to the development of several endoperoxide analogues, used mainly within combination therapies.[7] Despite this precaution, recent studies have reported a possible emergence of resistance against this drug and its derivatives.[8−10] This worrying observation emphasizes the importance of research for new drugs against malaria.[4,8−14]

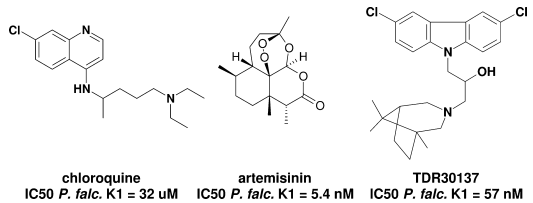


Figure 1. Structure and IC_{50} values of antimalarials against *Pf*-K1.[12−16]

As the identification and validation of new targets remains challenging,[17] most research programs use known antimalarials for pharmacophores[11] and rely on phenotypic screening.[18] Importantly the drug candidate should be efficacious against a large panel of *Plasmodium* species and show activity in a preclinical murine model. It should also be safe and stable in extreme conditions and require low cost of goods.[1]

The World Health Organisation (WHO) supported several programs to identify new chemical entities for the fight against malaria. Among several thousands of compounds screened within a public-private partnership collaboration with Tropical Diseases Research (TDR)−WHO,[19] the commercially available compound TDR30137 related to *N*-substituted carbazoles has emerged as a hit in a phenotypic in vitro screen against the chloroquine-resistant *P. falciparum* K1 (*Pf*-K1) in human red blood cells with an IC_{50} of 57 nM (Figure 1). Although the hit exhibited good in vitro potency, it did not show any activity in the in vivo *P. Berghei* mouse model used. Interestingly, carbazole scaffolds are found in nature with reported antimalarial activity.[20,21] Furthermore, synthetic carbazoles were also described as potential antimalarial agents,[22] Bax channel modulators,[23] and neuroprotective agents.[24,25] Thus, we thought that TDR30137 was a good starting point for a medicinal chemistry program. Herein, we report the stucture−activity relationship (SAR) study, the physicochemical and in

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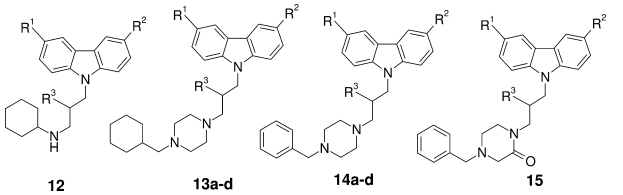
vitro and in vivo DMPK profiling, the in vivo efficacy, and finally the preliminary safety profile of a selected series of compounds that led quickly to the identification of an antimalarial lead.

In an effort to extend the knowledge around the new antimalarial hit TDR30137 (Figure 1), substructure searches and the synthesis of new analogues was performed to review the importance of the carbazole, the aromatic moiety, the spacer, and the amine type. Eighty compounds were selected, using substructure search, from Merck Serono screening library. Many modifications led to inactive compounds. Key potent features were found in the substituted carbazole linked to an amine via a floppy chain. Furthermore, 40 analogues were synthetised to refine SAR.

Compounds were evaluated through a parasitic growth assay at Swiss Tropical and Public Health Institute (Swiss TPH). This assay is based on the measurement of the incorporation of hypoxanthine after 3 days of incubation of a mixture of the evaluated drug, human red blood cells, and *P. falciparum*. SAR studies were performed by profiling analogues highlighting significant changes in terms of structure and behavior of substituents used. IC_{50}s were reported to compare and rank the differently substituted analogues (Table 1). Such compounds

Table 1. Structure−Activity Relationship Study



compd	R^1	R^2	R^3	*Pf*-K1 IC_{50}[a]	cpK_a[b]
12	Br	Br	OH	9 nM	10.0
13a	Cl	Cl	OH	16 nM	9.4
13b	Br	Br	F	106 nM	9.4
13c	Cl	Cl	=O	448 nM	9.4
13d	Cl	Cl	H	707 nM	9.4
14a	Br	Br	OH	27 nM	7.5
14b	Cl	H	OH	165 nM	7.5
14c	H	H	OH	275 nM	7.5
14d	H	H	OMe	3313 nM	7.5
15	Cl	Cl	OH	344 nM	5.3

[a]A 72 h assay in the presence of serum albumin. [b]Calculated pK_a predicted using pkcalc 4.1.0; CompuDrug, based on published work.[27]

were obtained via a two to five step straightforward synthesis from carbazole **1** and other cheap and readily available starting materials (see all details in Supporting Information). Among the most representative compounds for SAR evaluation, analogues **12** to **15** are summarized in Table 1.[26]

The removal of one or two halogens on the carbazole moiety (**14a** vs **14b** and **14c**, Figure 1) decreased activity of one log unit. In addition, the hydroxyl group and more particularly his hydrogen bound donor character seemed to be key for activity. Indeed, methyl ether **14d** vs compound **14c**, ketone **13c**, fluoro **13b**, and alkyl **13d** vs **13a** showed significant loss of activity. Finally, the basic character of the amine in the southern part seemed to play an important role in the activity on *Pf*-K1. For example, introduction of nonbasic functionality such as an amide (**15**) reduced the activity of one log unit. Other amides show the same tendency (data not shown). On the contrary, compounds **12** and **13a**, combining the presence of hydrogen bond donor group and optimized pK_a, showed excellent activity in low nanomolar range against *Pf*-K1.

In terms of selectivity, both compounds **12** and **13a** had an excellent in vitro potency against a panel of resistant *Pf* strains (K1, NF54, D6, W2, TM91C235, 7G8, and VIS).[28] Considering those promising in vitro results, the in vivo efficacy of the two compounds was assessed in a *P. Berghei* mouse model of infection. As compared to HuSCID model, which uses humanized mouse, the cheaper *P. Berghei* mouse model will ensure the possibility to have a greater amount of compounds tested in vivo.

As the chosen in vivo mouse model uses a *Plasmodium* strain different from the one used in the in vitro assays, (*P. berghei* vs *P. falciparum*, respectively), a correlation study was initiated, which confirmed the validity of the in vivo *P. Berghei* mouse model.[29]

Both compounds **12** and **13a** exhibited acceptable in vitro physicochemical and eADME characteristics for such lipophilic heterocyclic amines.[29] Interestingly, the low to medium intrinsic clearance measured translated into a medium in vivo clearance in mouse after intravenous administration at 1 mg/kg (∼15% and ∼30% of liver blood flow for compounds **12** and **13a**, respectively).[29] The intravenous volume of distribution was high for both amino compounds (13 L/kg and 15 L/kg for compounds **12** and **13a**, respectively) suggesting extensive distribution of the compound from the blood. Following oral administration, at 5 mg/kg, a favorable bioavailability of 50% was apparent, along with a long terminal half-life of 15 and 8 h for compounds **12** and **13a**, respectively. Furthermore, both compounds showed high C_{max} values (158 and 135 ng/mL for compounds **12** and **13a**, respectively). Compound **12** showed a higher AUC than **13a** (3753 h·ng/mL versus 1583), indicating a potential higher concentration in tissue as compared to compound **13a**. The pharmacokinetic profile was linear between oral doses of 3 and 100 mg/kg for compound **13a**, and between 3 and 30 mg/kg for compound **12**. Following these encouraging results in terms of clearance and bioavailability, an in vivo experiment in a mouse model infected with the *P. berghei* strain of the parasite was performed for both compounds.

The in vivo activity was assessed in the *P. berghei* mouse model using a 4-day once daily dosing regimen of the test compounds. The animals were treated orally on days 0−3 starting 4 h postinfection using dose groups of 3, 10, 30, and 100 mg/kg/day. Red blood cell parasitemia and plasma exposure were determined on day 4, 24 h after the last dose, followed by the monitoring of mean survival days (MSD) of the mice during 26 additional days.[33]

As shown in Figure 2A, the parasitemia of *P. berghei* infected mice was reduced in a dose-dependent manner with ED_{50} values of 11.4 and 34.5 mg/kg/day for compounds **12** and **13a**, respectively. By using the highest dose of 100 mg/kg/day, the total parasitemia could be reduced by 99.97% (**12**) and 99.93% (**13a**), therefore showing an almost complete disappearance of parasites in the mice after only 4 days of treatment.

In addition to the assessment of parasitemia reduction, the survival of the animals was also monitored during 26 additional days. Figure 2A shows that parasitemia was reduced by 38% after administration of 10 mg/kg/day of **12**; nevertheless, as shown in Figure 2B, no effect on the mean survival of the mice was seen. However, an increase of the dose from 10 to 30 mg/kg/day of compound **12** led to a significant increase of the

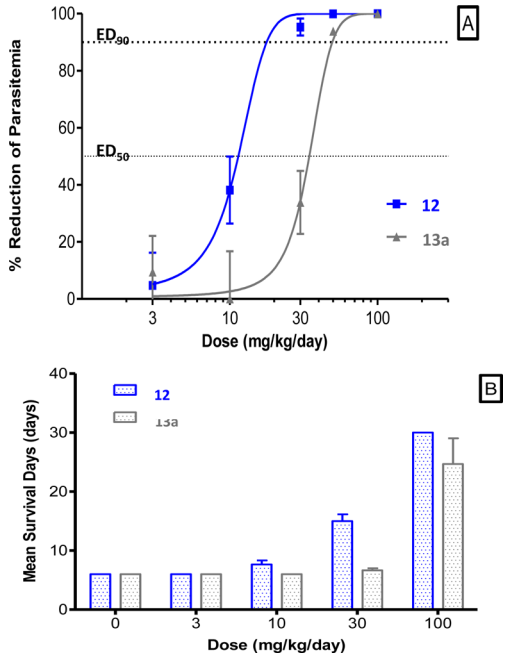


Figure 2. Dose dependency of activity of compounds **12** and **13a** against *Pf*-K1. (A) Dose response curve of compounds **12** (blue) and **13a** (gray) in the *P. berghei* infected mice (4 day-treatment, po). (B) Mean survival days (MSD) of *P. berghei* infected mice after 4-day treatment po.

mean survival days (MSD) of the mice. Concerning compound **13a**, a minimum dose of 100 mg/kg/day is needed to reach a real increase of MSD. This dose of 100 mg/kg/day led to the survival of the mice during 30 days by using compound **12** after oral administration.

These in vivo experiments led to the selection of compound **12** as being the most promising compound, considering the increase of the survival of the animals treated by 100 mg/kg/day, as compared to its analogue **13a**.[30]

Safety profile is of extreme importance while considering the situation of such patients, living mainly in areas with limited medical supervision. First genotoxicity and mutageniticity assays were performed. Racemate **12** was found negative in micronucleus and AMES in vitro assays, including under metabolic conditions.

Then, in order to assess the cardiac toxicity of compound **12**, the first safety assays performed were focusing on human ether-a-go-go related gene channel (hERG channel). The inhibition of this cardiac ion channel can lead to cardiotoxicity, which has been reported for several antimalarial compounds (e.g., Halofantrin, Chloroquine, K_i = 7.5 μM; Mefloquine, K_i = 1.9 μM).[31] The activity of compound **12** toward hERG was high, showing an inhibition of the channel of 99% at 10 μM. It is noteworthy that compound **12** did not display any inhibition against any other cardiac ion channels profiled (hNa$_V$1.5, K$_V$4.3, hCa$_V$1.2, hK$_V$1.5, hHCN4, and hKir2.1), suggesting that only hERG has to be taken into account.[33]

As every biological target, this activity on the ion channel could be linked to the stereochemistry of the compounds tested. To assess if the toxicity risk was only linked to one of the two enantiomers, the enantiopure compounds were prepared in a 3-step synthesis.[26]

The profiling of both enantiomers of compound **12** showed that compounds **18a** and its enantiomer **18b** had the same activity against *Pf*-K1, hERG, and the panel of CYPs than the racemate **12**.[29] Thus, the profiling of the two enantiomers did not show any improvement of safety parameters as compared to the racemate **12**. Those parameters and mainly hERG inhibition should be the identified liability to solve, thanks to medicinal chemistry design of new compounds. The design of novel compounds would hence be based on the knowledge of hERG pharmacophore to decrease the activity on the channel.

The described pharmacophore for hERG inhibition (Figure 3A) shows that compounds **12**, **18a**, and **18b** contain chemical fragments that displayed physicochemical properties inhibiting the activity on the channel (Figure 3B).[32,34]

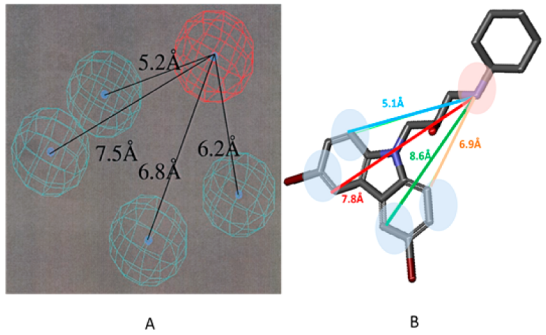


Figure 3. hERG pharmacophore vs compound **12** structure. (A) hERG pharmacophore as depicted in the literature.[34] The blue spheres depict lipophilic areas and the red sphere represents an ionizable center. Reproduced with permission from ref 34. Copyright 2002 American Society for Pharmacology and Experimental Therapeutics. (B) Calculation of distances between the lipophilic centers (blue) and basic centers (red) of compound **12**.

The distance between the lipophilic areas and ionic center of compounds **12**, **18a**, and **18b** corresponds to the described pharmacophore, predicting a high affinity of the compounds to the ion channel.

Despite the great in vitro and in vivo activity of compounds **12**, **18a**, and **18b** against the parasite, current work for optimization alleviating the hERG inhibition is evaluated.

Thanks to a Private Public Partnership between Merck Serono and WHO, a HTS campaign led to the identification of an interesting hit. The screening of about 80 analogues from Merck Serono screening library and a focused medicinal chemistry program helped to build a SAR, leading to the identification of compound **12**, with in vivo efficacy. Nevertheless, safety evaluation revealed hERG inhibition, which could be challenging for the development toward a preclinical candidate. In parallel, a backup strategy, developing a different series in terms of structures and profiles, has been put in place relying on drastic changes of physicochemical properties[35,36] of the series, which will be shortly communicated.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and analytical data of intermediates and exemplary compounds. Details for Pf-K1 in vitro growth assay. *P. berghei* in vitro growth assay, microsomal stability, CYP inbibition in vitro assay, and rodent pharmacokinetic studies. *P. berghei* in vivo assay. hERG patchclamp assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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All authors have given approval to the final version of this manuscript.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

CL, *in vivo* clearance; CLint, intrinsic clearance; CYP, cytochrome P450; ED_{50}, 50% efficacy dose; Fz, bioavailability; hERG, human ether-a-go-go related gene; HTS, high throughput screening; IC_{50}, 50% inhibitory concentration; IC_{90}, 90% inhibitory concentration; MSD, mean survival days; *P. berghei*, *Plasmodium berghei*; *Pf*, *Plasmodium falciparum*; PK, pharmacokinetic profile; *po*, per os; SAR, structure−activity relationship; Vss, volume of distribution at steady state; WHO, World Health Organization

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(28) Data are detailed in the Supporting Information, in the paragraph "In vitro anti malarial activity (IC_{50} and IC_{90}) for (**12**) against a panel of *Plasmodium falciparum* strains."
(29) See Supporting Information in additional information chapter.
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