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Letter

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Case Study of Small Molecules As Antimalarials: 2-Amino-1-phenylethanol (APE) Derivatives

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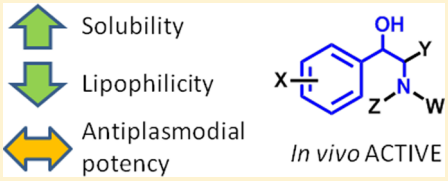
Supporting Information

ABSTRACT: Antiparasitic oral drugs have been associated to lipophilic molecules due to their intrinsic permeability. However, these kind of molecules are associated to numerous adverse effects, which have been extensively studied. Within the Tres Cantos Antimalarial Set (TCAMS) we have identified two small, soluble and simple hits that even presenting antiplasmodial activities in the range of 0.4−0.5 μM are able to show in vivo activity.



KEYWORDS: *Aminoalcoholes, malaria, tres cantos antimalarial set, TCAMS, mefloquine*

Clinical resistance against artemisins has already been reported. Artemisinins are the mainstay of Artemisinin Combination Therapies (ACTs), which currently are the first line of antimalarial treatment recommended by WHO to treat *P. falciparum* malaria. As a consequence, there is an urgent need to develop novel antimalarial drugs as potential replacements for artemisinins.[1] Aiming at disease eradication, it would be enormously advantageous that the new drugs were active also against gametocytes (the sexual stage responsible for transmission of the parasite to mosquitoes) and hypnozoites (dormant liver stages responsible for the malaria relapses characteristic of *P. vivax*).[2,3]

Looking back in time we can find a similar situation in malarial chemotherapy. In 1970s resistance to chloroquine (**1**), the main antimalarial drug at the time, was dramatically increasing.[4] Chloroquine resistance was observed first in Asia (Thailand in 1957) and South America (Colombian−Venezuelan border in 1959) and, almost two decades later, in Africa (Kenya and Tanzania in 1978). In the period 1960−1970s, the Experimental Therapeutics Division of the Walter Reed Army Institute of Research (WRAIR) developed the largest drug discovery program ever addressed in malaria history because of the Vietnam War where up to 1% of US troops suffered from malaria. An extensive screening campaign was pursued and 250,000 potential antimalarial compounds were screened. Two related compounds presenting a phenyl-amino-alcohol scaffold were identified: compounds 142,490 (mefloquine (MQ, **2**)-lariam) and 171,699 (halofantrine (**3**)-halfan) (Figure 1). These compounds were marketed without phase III trials due to the special historical conditions in which they were developed.

In early 2000s, three randomized controlled trials in healthy populations confirmed that aminoalcohols can cause physio-

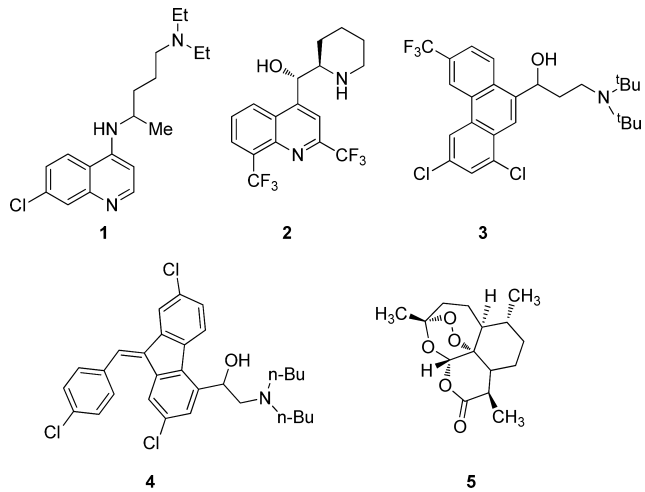


Figure 1. Exemplars of common antimalarials.

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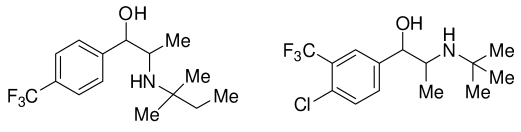
logical illnesses. Mefloquine has central nervous system and gastrointestinal tract toxicity, whereas halofantrine has cardiotoxic effects.[5,6] Despite many unknowns, aminoalcohols have been the subject of intense research over the last 40 years. A third representative of this chemical series, lumefantrine (**4**), is currently used in combination with artemether (**5**) for first-line acute therapy. This combination, marketed as Coartem, represents 75% of all ACT treatments used in the clinic. Coartem provides a rapid relief of symptoms due to the fast killing mode of action of Artemether and prevents recrudescence due to the long half-life (4 days) of lumefantrine.

Representatives of the 2-amino-1-phenylethanol (APE) series have been found in the GlaxoSmithKline's Tres Cantos Antimalarial Set (TCAMS) (**6** and **7**, Figure 2).[7,8] These molecules were attractive since they were structurally simpler than "classical" aminoalcohols and showed amenable physicochemical properties (Table 1).



6 **7**

Figure 2. TCAMS-APES derivatives selected.

The compounds **6** and **7** were found efficacious in vivo against *P. berghei* in a screening assay recently published.[13] These compounds were initially deprioritized for further progression after in silico filtering of the TCAMS[9−11] because compounds **6** and **7** had an amino-phenyl-ethanol core similar to mefloquine.[12] However, compounds **6** and **7** clearly reduced parasitemia in *P. berghei*-infected mice with respect to the vehicle group (64 and 76%, respectively) upon qd oral administration during two consecutive days despite presenting a relatively high in vitro IC_{50} (Table 1). Interestingly, compounds **6** and **7** showed a fast absorption and high blood exposure upon single dose oral PK analysis in the in vivo screening conditions (Figure 3). These data correlated well with a good solubility and low intrinsic clearance (Cl_i) in liver mouse microsomes (Table 1). As a preliminary measure of toxicity we decided to evaluate these compounds in a panel of mammalian cell lines (HepG2, L1210, Neuro2A, H9c2(2−1), and MDCK, Table 1), and the results showed low risk of cytotoxicity. From a developability point of view, the compounds did not present any issues related with 3A4CYP inhibition (>30 μM).

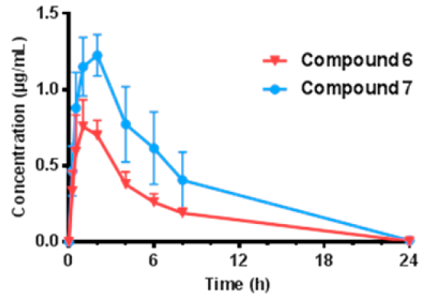


Figure 3. Oral PK profile for compounds **6** and **7**.

These molecules display a robust in vitro parasitological profile: parasite cultures treated with compound **6** at 10 × IC_{50} showed a pycnotic phenotype only 24 h after commencing exposure. This phenotype is characteristic of compounds with a fast-killing antimalarial mode of action as is the case of chloroquine or artemisinin.[14] Compounds with a slower mode of action such as atovaquone, a mitochondrial bc1 complex inhibitor, exerts its antimalarial effect later in the life-cycle and stop cultures in the trophozoite stage. An additional result supporting a fast speed of action for these compounds is the reduction of more than 4 orders of magnitude in the number of viable parasites observed when cultures are exposed to **6** for 72 h at 10 × IC_{50}. A similar reduction of viability occurs with chloroquine treated parasites.

Molecules are equally potent in vitro when assayed against the wild-type 3D7 strain and resistant strains such as HB3 (moderately resistant to pyrimethamine), Dd2 (chloroquine and pyrimethamine resistant), TC08 (pyrimethamine resistant), T9/94 (chloroquine resistant), V1/S (chloroquine and pyrimethamine resistant), FCR3 (chloroquine, atovaquone, and cycloguanil resistant), Tm90C2A (chloroquine and pyrimethamine resistant), and Tm90C2B (chloroquine, pyrimethamine, and atovaquone resistant), with potencies in the nanomolar range (Table 1).

To determine the 3D-structure−activity relationship (SAR) we first started by determining the optical purity (not showed in Chembl database) of the hits by using a chiral solvating agent (Pirkle's reagent,[15] ((*S*)-(+)-1-(9-anthryl)-2,2,2-trifluoroethanol)). This experiment revealed that the hit was a mixture of enantiomers. This result was confirmed by c-HPLC.

The enantiomers of **6** and **7** (**13**−**16**, Table 2) were separated using preparative c-HPLC and their stereochemistry assigned by ab initio vibrational circular dichroism (VCD), an analytical technique for determining absolute stereochemistry.[16] Experimental and theoretical data shown in Figure 4 for **13** and **14**

Table 1. Complete Profile for 6 and 7

	compd **6**	compd 7
LE, LLE, M_w	0.40, 2.42, 289	0.43, 2.75, 309
chromlogD 7.4/PFI	3.8/4.8	4.5/5.5
Pf IC_{50} μM (3D7A)	0.4	0.5
efficacy (mg/kg) *P. berghei*	ED_{50} < 50	ED_{50} < 50
in vitro CL (mL/min·g), $T_{1/2}$ (min) (mouse)	0.5, >30	0.3, > 30
protein binding mouse plasma (%)	91.7	95.5
P450 3A4 (vivid red/vivid green) μM	>50/50	>50/31.6
solubility (μg/mL) PBS/FaSSIF/FeSSIF/SGF	N.D.	>600, >600, >600, >600
Tox_{50} (μM) (HepG2, Jurkat, CHP212, MDCK, H9C2(2−1))	>50, >50, >50, >50, >50	48.8, >50, 48, >50, >50
PRR (parasite reduction rate) (72 h)	fast	N.D.
Ratio 3D7/TC08, HB3, T9/94, Dd2, V1/S, FCR3, Tm90C2A, Tm90C2B	∼1	∼1

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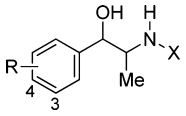
Table 2. Key 3D-SAR Data



compd	configuration	X	R_3	R_4	IC_{50} (μM) *Pf* 3D7A
6	*rac-threo*	*tert*-pentyl	H	CF_3	0.4
8	*rac-erythro*	*tert*-pentyl	H	CF_3	>5
9	*rac-threo*	*tert*-pentyl	Cl	Cl	0.2
10	*rac-erythro*	*tert*-pentyl	Cl	Cl	3.9
11	*rac-threo*	*tert*-butyl	Cl	Cl	0.9
12	*rac-erythro*	*tert*-butyl	Cl	Cl	>5
7	*rac-threo*	*tert*-butyl	CF_3	Cl	0.5
13	(*S*,*S*)	*tert*-pentyl	H	CF_3	1.4
14	(*R*,*R*)	*tert*-pentyl	H	CF_3	0.5
15	(*S*,*S*)	*tert*-butyl	CF_3	Cl	0.6
16	(*R*,*R*)	*tert*-butyl	CF_3	Cl	0.3
17	(*S*,*S*)	*tert*- pentyl	Cl	Cl	0.5
18	(*R*,*R*)	*tert*- pentyl	Cl	Cl	0.4

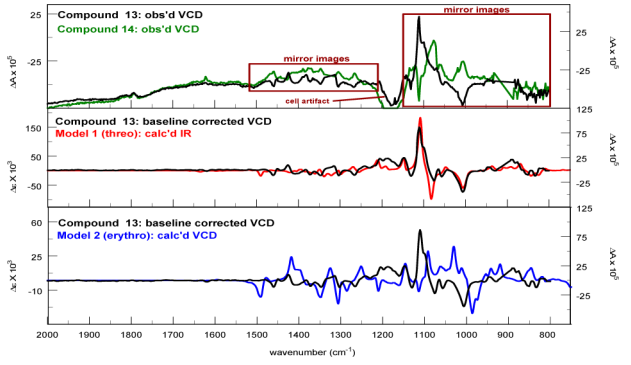


Figure 4. Blue line corresponding to enantiomer E1 (**13**) and red line corresponding to enantiomer E2 (**14**) of **6**.

illustrate how VCD analysis was applied to various representatives of the APE series. In the top panel, VCD spectra observed for **13** and **14** in the fingerprint region (uncorrected for baseline artifacts) are compared. The spectral patterns in the two highlighted regions are mirror images, confirming these molecules are enantiomers. The baseline corrected VCD spectrum of **13** is compared to VCD spectra calculated for (a) Model 1, the *threo* model with (*S*,*S*) configuration, and (b) Model 2, the *erythro* model with (*R*,*S*) configuration in the middle and bottom panels, respectively. While the spectrum of Model 1 appears to be a good match with **13**, the one calculated for Model 2 clearly is not, supporting assignment of Model 1 as the correct stereoisomer of **13**. This assignment was confirmed using CompareVOA (BioTools, Inc. Jupiter, FL), an algorithm for quantifying the agreement between experimental and theoretical VCD spectra. Results from the CompareVOA analysis indicated that only an assignment based on Model 1 would be reliable, with an estimated confidence limit >99% (as opposed to Model 2, with an estimated confidence limit <75%). On the basis of these findings, **13** was assigned as the (*S*,*S*) enantiomer and **14** as the (*R*,*R*) enantiomer (Figure 4).

This result was confirmed using nuclear Overhauser effect (NOE) experiments to determine the relative configuration and the formation of chiral esters with the (*R*) and (*S*) enantiomers of the methoxyphenylacetic acid (MPA) and comparison of the $\Delta\delta^{RS}$ signs NMR spectra of the resulting diastereomers [(*R*)- and (*S*)-MPA esters].[17] The assignment is based on the existence of a correlation between the chemical shifts of those derivatives and their stereochemistry (absolute configuration) (Figure 5).

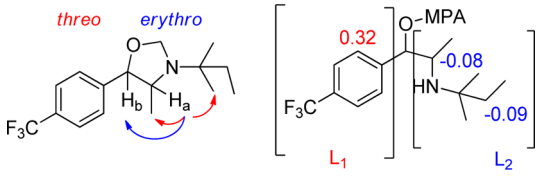


Figure 5. Key NOE for signals for oxazolidine derivative (left).[18] Δ^{T1T2} values (ppm, 298 K, $CDCl_3$) obtained for the bis-(*R*)-MPA and bis-(*S*)-MPA esters (right).[19]

To understand the 3D-SAR, we started by comparing the antiplasmodial activity for the *threo* and *erythro* derivatives of three compounds (**6**, **9**, and **11** versus **8**, **10**, and **12**, respectively; Table 2). We observed that in all the cases the *threo* racemic mixture ((*R*,*R*) and (*S*,*S*)) was significantly more potent than the *erythro* one ((*R*,*S*) and (*S*,*R*)). However, when we analyzed the *threo* enantiomers separately for **6**, **7**, and **9**, no clear differences can be argued (pairs **13**−**14**, **15**−**16**, and **17**−**18**, Table 2, respectively). In conclusion, as result of this SAR study shown in Table 2 we can say that the series presents a clear preference for the *threo* derivatives, while no clear differences are detected between the enantiomers. This is an important difference with, for example, mefloquine in which the four diastereomers present the same in vitro potency although the difference in the PK supports the use of the *erythro* isomers instead of the *threo*.[20]

In fact, with the objective of rationalizing the stereospecificity observed for the TCAMS aminoalcohols, we used Mefloquine as model system by means of flexible alignments using the MOE program.[21] The flexible alignment of the (*R*,*R*) isomer of **6** with +(*R*,*S*) mefloquine gave a good superimposition (Figure 6a). Similar good superimposition was observed between the (*R*,*S*)

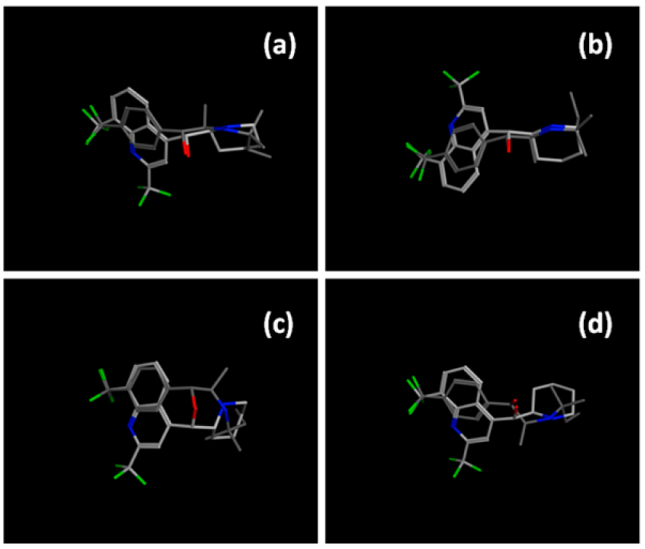


Figure 6. Flexible alignment of (*R*,*R*) and (*R*,*S*) isomers of **6** with mefloquine. Converged superposition of the (*R*,*R*) isomer of **6** with +(*R*,*S*) mefloquine; (b) superposition of the (*R*,*S*) isomer of **6** with +(*R*,*S*) mefloquine; (c) superposition of the (*R*,*R*) isomer of **6** with +(*S*,*R*) mefloquine; (d) superposition of the (*R*,*S*) isomer of **6** with +(*S*,*R*) mefloquine. Mefloquine carbon atoms are shown in light gray, **6** in dark gray. No hydrogens are shown for the sake of clarity.

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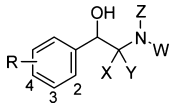
Table 3. Key 2D SAR Data



compd	configuration	R_2	R_3	R_4	X	Y	Z	W	IC_{50} (μM) *Pf* 3D7A
19	*threo*	H	H	CF_3	Me	H	H	H	>5
20	*threo*	H	H	CF_3	Me	H	*tert*-pentyl	Me	>5
21	*threo*	H	H	CF_3	Me	H	Phe	H	5
22	*threo*	H	H	CF_3	Me	H	cyclohexyl	H	5
23	*racemic*	H	CF_3	Cl	H	H	*tert*-butyl	H	4.3
24	*racemic*	H	Cl	Cl	H	H	*tert*-butyl	H	>5
25	*racemic*	H	Cl	Cl	H	H	cyclohexyl	H	0.72
26	*racemic*	H	Cl	Cl	H	H	cycloheptyl	H	0.16
27	*racemic*	H	Cl	Cl	H	H	cyclooctyl	H	0.07
28	*racemic*	H	Cl	Cl	H	H	Bn	H	0.7
29	*racemic*	H	Cl	Cl	H	H	2-Me-naphtalene	H	>5
30	*racemic*	H	H	H	Me	H	*tert*-butyl	H	>5

isomer of **6** and +(*R*,*S*) isomer of mefloquine (Figure 6b). This should indicate that the origins of stereospecificity of our aminoalcohol series and mefloquine are different. On the contrary, flexible alignments of both the (*R*,*R*) and (*R*,*S*) isomers of **6** to −(*S*,*R*) mefloquine gave much worse alignments (Figure 6c,d, respectively). Therefore, again similar superimposition behavior is seen for the two isomers of (**6**), but in this case, the observed alignments are not as good as with the +(*R*, *S*) isomer.

Regarding the 2D-SAR, these are the key conclusions: we know that free-amine compound (**19**) is inactive (Table 3) and that secondary amines (**6**) are preferred versus tertiary (**20**) (Table 3). In fact, for compounds related to **6**, only *n*-alkyl derivatives have resulted inactive (either phenyl (**21**, Table 3) as well as cyclo-alkyl (**22**, Table 3) derivatives presented an *Pf*IC_{50} of 5 μM). A loss in activity was also observed in the demethylated derivative of **7** (**23**, Table 3). Another critical observation is the change in the SAR when the 3,4-di-chloro substitution is used (Table 3) at the left-hand side of the molecule instead of the *p*-CF_3 (**6**) or the 3-CF_3-4-Cl (**7**). In this case, the demethylated compound (**24**, Table 3) is inactive but the cyclo-alkyl derivatives (**25**−**27**, Table 3) present a low IC_{50}. In fact, while increasing the size of the cycle a lower IC_{50} was obtained, although it is not related to a simple increase in lipophilicity as aromatic moieties (**28** and **29**, Table 3) present IC_{50}s of 0.7 and >5 μM. In conclusion, results shown that opportunities for improving potency are possible without increasing lipophilicity. Finally, this first SAR study has revealed that the aromatic ring must be substituted to observe antiplasmodial activity (**30**, Table 3).

To finalize this early profiling of this chemical series we decided to compare the TCAMS compound with the closed aminoalcohol, mefloquine. Obviously both contain the amino-alcohol scaffold but from a 2D perspective, mefloquine presents a more lipohilic aromatic moiety and a cycle-tertiary amine, while the TCAMS compounds present a phenyl moiety and a linear secondary amine. We have already discussed the differences from a 3D point of view prior. Finally, two assays were carried out in order to delve into this subject: we compare their capacity to inhibit the heme-polymerization and their safety profile by comparing their promiscuity against a panel of receptors, ion channel, enzymes, and transporters.

It is well-known that *P. falciparum* detoxifies host hemoglobin-derived ferriprotoporphyrin IX (Fe(III)PPIX) in an acidic digestive vacuole mainly by converting it to hemozoin. It is not clear what is the mechanism of hemozoin formation, but Pisciotta et al.[17] have shown that it could be formed in a lipid body dubbed a lipid nanosphere inside the vacuole. Evidence for the mechanism of action of the aryl methanols such as quinine (QN), mefloquine, and halofantrine (Hf) is less clear-cut. These drugs have been shown to form complexes with Fe(III)PPIX and to inhibit formation of β-hematin, the synthetic counterpart of hemozoin, and also to influence the growth of β-hematin crystals. The β-hematin formation assays revealed that mefloquine is a potent inhibitor (IC_{50} 12.15 μM), while **6** and **7** do not present any activity up to 100 μM.

In vitro pharmacological profiling involved the screening of compounds against a broad range of human targets (receptors, ion channels, enzymes, and transporters) that are distinct from the intended therapeutic target (or targets) in order to identify specific molecular interactions that may cause adverse drug reaction in humans. This panel of assays was done with mefloquine (**2**), the *threo* mixtures of both hits (**6** and **7**), and enantiomers of **7** (**15** and **16**). Although some key differences were found (i.e., inhibition of serotonin), the safety profile for all the compounds assayed is very similar (Figure 7), and some

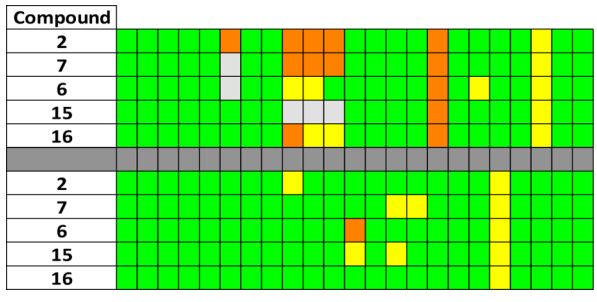


Figure 7. Bit map of selected key compounds (*y* axis) when tested against a panel of human receptors, enzymes, ion channels, and transportes (*x* axis). Color code: green/orange/red, low/medium/high risk. Light gray means no data.

alerts were detected. These alerts are related to cardiovascular system (CVS) and central nervous system (CNS), like Ca and hERG channels and some serotonin isoforms. The cleanest profile is of one of the isomers, **16**, but this still shows some alerts related to CNS and CVS. These alerts anticipate similar issues

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during the development of these compounds, although further analysis should be made.

In conclusion, in this communication we show a case study of small molecules as antimalarials, which present in vivo efficacy when administered orally. The preliminary SAR shows opportunities to increase the potency, but the safety profile does not preclude the same side-effects as mefloquine. However, this case study is very encouraging to continue the quest for a differential antimalarial regarding the physicochemical profile.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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