Optimization of compounds from the pyrazole series for malaria treatment

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INTRODUCTION:

Malaria is a disease that has been impacting millions of victims annually throughout the world. In 2022, about 249 million malaria cases had been registered in the world, of which 150 thousand were only in Brazil, an endemic country and one of the most important epicenters of contamination in America. In the same year, Brazil, Venezuela, and Colombia were responsible for 73% of cases reported in America, most resulted from *P. vivax* infection – 72% of cases[1]. Malaria is endemic in 85 countries and regions around the globe, with Africa being its major center, with 93,6% of cases and 95,4% of the deaths registered in 2022[1]. Regarding the deaths, 78,1% are from infants under five years old[1], resulting in an estimative of one death per minute[2]. The disease is also considered risky for pregnant women[1].

The family of *Plasmodium* is responsible for the human malaria contamination. It is subdivided into five main strains: *P. falciparum, P. vivax, P. ovale, P. malarie* and *P.knowlesi.* Of those, *P. falciparum* is the most associated with severe malaria and deaths[3]. Throughout the past decades, resistance has been noticed in different intensities against available treatments. One example is the resistance encountered in *P. falciparum* against artemisinin derivatives, compounds found in the combined therapies that represent the most important line of treatment used nowadays[4].

Despite the growing resistance among the parasites, chemotherapy is still one of the most efficient and effective methods against malaria infections. Combined therapies that use an artemisinin derivative and another long-duration antimalarial are widely employed. On the other hand, chloroquine is an example of an easy-synthesis drug still used up north in Brazil, given the absence of resistant strains in that region. Finally, primaquine is another representative antimalarial, responsible for eliminating hypnozoites in cases of *P. vivax* infection, which is accountable for the incidence noticed in those cases.

To fight the failure of most known therapies due to growing resistance, MMV—Medicines for Malaria Venture—was founded in 1999 as an institution that aims to reduce the impacts generated by malaria using high-level research, development, and distribution of adequate and accessible antimalarial medications. With partners all around the globe and a world-class team, MMV is present throughout the drug discovery process, being responsible for fifteen already-in-use medicines[5].

METHODOLOGY:

This project is based on optimizing compounds from the *MMV2301* series. The main goal is to design and synthesize prototypes from the series hit provided by *MMV* based on potency and pharmacokinetics data through *SAR—Structure-Activity Relationship*. The hit is found by phenotypic screening on virtual libraries owned by *MMV*, which is the first step of the drug discovery process. The *MMV2301* has about forty compounds synthesized.

To be classified as a pre-clinical candidate, which is the target compound of the project, a molecule must respect the parameters established by *MMV*. Given the desire to produce an orally administrated single-dose medicine, the ranges are tighter to provide a drug efficient enough to play its role properly. The potency must be 10 nM or lower, while the solubility must be higher than 50 µM (**Figure 1**). The logD must be lower than 3 to provide adequate permeability in human and parasite cells. Pharmacokinetics parameters such as those related to clearance (*CL*), half-life time ($t_{1/2}$), and bioavailability (*F*) should be used to guarantee a drug that stays as long in the organism as necessary to allow a single dose administration without affecting its efficiency. Drug toxicity is also a concern, as the hERG channel inhibition must be kept greater than 10 µM (**Figure 1**).

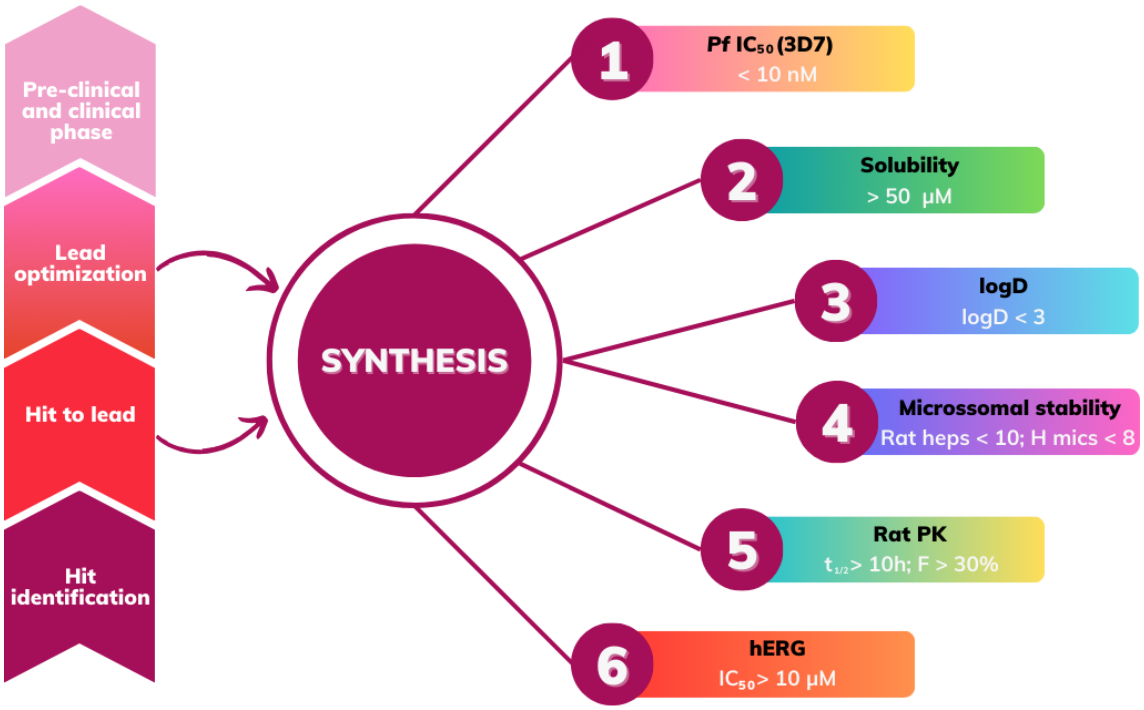


Figure 1: Drug discovery process and MMV goal parameters for a pre-clinical candidate.

Beyond the previously presented parameters, some based on literature are also used to enlighten the development process. An example is Lipinski's "rule of five," which is determined by statistical data and supplies critical parameters for oral drug development[6]. Software such as *StarDrop* and *SMARTCyp* are also essential allies in an era where artificial intelligence (*AI*) and machine learning become powerful tools in drug design.

RESULTS AND DISCUSSION:

The analogues of the *MMV2301* series were synthesized employing a parallel synthesis approach with a convergent nature. The final compound is achieved by a nucleophilic attack of the amine to the carbonyl, originating an amide (step E, **Figure 2**). The *RHS* (*Right-Hand Side*) of the molecule is a primary amine, while a carboxylic acid represents the *LHS* (*Left-Hand Side*) moiety.

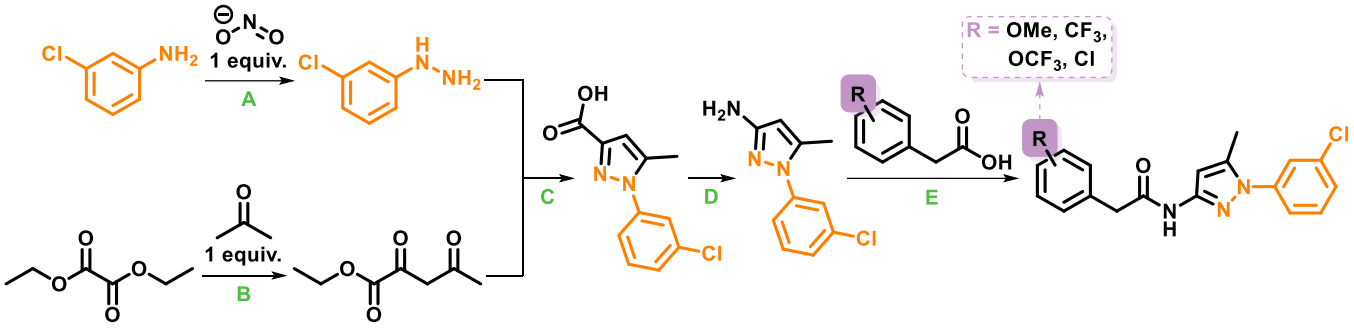


Figure 2: simplified synthetic route for the *MMV2103* analogues[a].

[a]Reagents and reaction conditions to the synthesis of *MMV2103* analogues: **A-)** $NaNO_2$ (1.0 equiv.), $SnCl_2$ (5.2 equiv.), HCl, EtOH, *yield* = 85%; **B-)** $Na_{(s)}$ (1.3 equiv), EtOH, 0°C, 2h, *yield* = 80 %; **C-)** i: DIPEA (1.1 equiv.), NaOH (5 equiv.), EtOH, 60°C, 3h; ii: EtOH:H_2O, 80°C, *yield* = 30%; **D-)** TEA (3.5 equiv.), DPPA, toluene, 50°C, *yield* = 50%; **E-)** DIPEA (2 equiv.), OHBt (1 equiv.), EDC (1 equiv.), DMF, rt, 18h, *yield* = 58%.

The *RHS* follows a parallel approach with hydrazine and pentanoate production. For the hydrazine, the synthesis is started with the commercial chlorinated aniline, which is converted to the desired hydrazine by reaction with sodium nitrite (step A, **Figure 2**). At the same time, the ethyl 2,4-dioxopentanoate is being produced through a reaction that involves acetone with metallic sodium and diethyl oxalate (step B, **Figure 2**).

With both fragments synthesized, the route converges to give a carboxylic acid via a cyclization reaction in basic pH, known as the Knorr reaction (step C, **Figure 2**). The acid function is then converted into an amine using Curtius' rearrangement (step D, **Figure 2**), giving the molecule's *RHS*.

To synthesize the desired series prototype, the amine produced is reacted with a commercially available carboxylic acid. In ideal conditions, the nucleophile attacks the carbonyl, and the product can be isolated (step E, **Figure** 2). The carboxylic acid's "R" fragment is variable in both nature and connectivity to the aromatic ring. It is also a portion where the *SAR* is explored.

Once the compounds are synthesized and have a 95% or higher purity level, they are submitted to tests in partners' laboratories for biological analysis. **Table 1** shows the result of the *ADME* (*Absorption, Distribution, Metabolism, and Excretion*) Tier 1 scan for the series hit and three prototypes. The "R" fragment representing each change on its respective position is illustrated (methoxy for the hit, chloride, OCF_3, and trifluoromethyl for the prototypes). Those compounds have a considerable potency against *P. falciparum* (strain *3D7*), being of 0.69 µM for the hit, 0.78 µM to the one with the Cl, 0.2 µM when the R group is represented by OCF_3 and, finally, 0.45 µM to the analogue with the CF_3 moiety. These potencies, though, are still far from the minimum 10 nM required (**Figure 1**). Despite the relevance usually associated with potency during the drug discovery process, it is not the only important parameter while making a druggable molecule[7]. Absorption, distribution, excretion, and metabolism may also be

considered when aiming for an oral drug, given that they are decisive in its pre-clinical and clinical success.

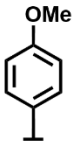
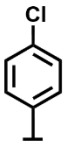
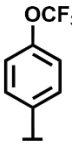
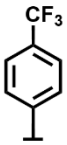
	OMe	Cl	OCF_3	CF_3
				
IC_{50} - Pf3D7 (µM)	0.69	0.78	0.20	0.45
logD	4.11	4.10	4.93	5.78
Solubility - pH 7.4 (µM)	13.50	<2.50	<2.50	<2.50
hMics - CLintMIC (µL/min/mg)	18.80	16.80	<3.50	3.92
Rat - CLintHEP (µL/min/10^6)	21.90	59.30	20.92	18.14

Table 1: potency and pharmacokinetics data for the hit and another three analogues from the *MMV2103* series.

The logD, a determinant parameter for oral drug absorption and distribution, is out of the ideal range for both the hit and all the represented analogues. Though all show undesired values, the compounds with trifluoromethyl have the highest data, of 4.93 when the CF_3 is connected to oxygen and 5.78 when it is directly bonded to the ring (**Table 1**). Once this molecule characteristic is related to one's interaction with apolar molecules, compounds with such a high logD may manifest an interaction too strong with the cell's proteins and lipids when tested in an *in vivo* system. The higher the logD, the higher the molecule interaction with apolar compounds such as proteins like albumin, which implies a correlation between the logD and albumax binding data associated with blood drug distribution. It also affects the solubility manifested by those compounds, which, as seen by the data reported (**Table 1**), is extremely low at plasma pH. A compound's logD can be decreased by modulating the presence of aromatic rings, halogens *(e.g.*, CF_3, Br, and Cl), as well as alkyl chains since those tend to increase this molecule property.

In terms of clearance, all molecules have undesired metabolic stability in rat hepatocytes cells, being especially high for the one with the chloride. Even so, those with OCF_3 and CF_3 fragments have great stability when tested in the human microsome, representing an improvement in the analogues concerning the rate at which those are metabolized. Clearance is a determinant factor for a long-lasting drug as desired, also directly proportional to the molecule's half-life time.

The series' compounds have various hot spots for CYP oxidation in their structure, confirmed by literature data and software projection[6]. The CYP450 superfamily is the main responsible for xenobiotic oxidation and directly correlates with drug metabolic stability. Nevertheless, CYP enzymes are not the only ones involved in drug metabolism. Amides often undergo hydrolysis, specially driven by serine hydrolases, which can explain the metabolic instability noticed in the series members. Furthermore, the aromatic amines released are frequently classified as toxic metabolites, which becomes a concern in drug discovery[8].

CONCLUSION:

The synthetic route is appropriate for enabling the synthesis of multiple prototypes. About forty analogues have been synthesized through the synthetic route presented, some with minor variations. The parallel-convergent route has a global yield of 5.9%.

The chemical series warrants different modifications that open possibilities for exploring diverse molecule designs as antimalarials. However, though manifesting some potency against the principal malaria agent, *P. falciparum*, the series' analogues display poor pharmacokinetics parameters that are key for a preclinical candidate profile. The high logD, poor solubility, and, most especially, the high clearance manifested for most of the series prototypes led to the series being discontinued.

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