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Clofazimine analogs with antileishmanial and antiplasmodial activity

Anna Barteselli [a], Manolo Casagrande [a], Nicoletta Basilico [b], Silvia Parapini [c], Chiara M. Rusconi [a], Michele Tonelli [d], Vito Boido [d], Donatella Taramelli [c], Fabio Sparatore [d], Anna Sparatore [a,*]

[a] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy
[b] Dipartimento di Scienze Biomediche Chirurgiche e Odontoiatriche, Università degli Studi di Milano, Via C. Pascal 36, 20133 Milano, Italy
[c] Dipartimento di Scienze Farmacologiche e Biomolecolari—DiSFeb, Università degli Studi di Milano, via C. Pascal 36, 20133 Milano, Italy
[d] Dipartimento di Farmacia, Università di Genova, Viale Benedetto XV 3, 16132 Genova, Italy

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ABSTRACT

A set of novel riminophenazine derivatives has been synthesized and evaluated for in vitro activity against chloroquine–sensitive (CQ-S) and chloroquine–resistant (CQ-R) strains of *Plasmodium falciparum* and against different species of *Leishmania* promastigotes. Most of the new compounds inhibited the growth of *Leishmania* promastigotes as well as CQ-S and CQ-R strains of *P. falciparum* with IC_{50} in submicromolar range, resulting in the best cases 1–2 orders of magnitude more potent than the parent compound clofazimine.

1. Introduction

Malaria is a major life-threatening parasitic disease in humans worldwide. Despite all the efforts and investments done over the last decade to roll back the disease, malaria was still responsible for 207 million clinical cases and 627,000 deaths in 2012, most of them children living in sub-Saharan Africa.[1] The emergence of resistant strains of *Plasmodium falciparum* (*P.f.*) even to the most recent combinations of antimalarial drugs,[2] including the artemisinin derivatives,[3] makes urgent the development of new anti-plasmodial molecules using different strategies.

After malaria, leishmaniasis is the second most important parasitic infection worldwide for mortality in humans. It is transmitted by the bite of a sand-fly infected by a flagellate protozoan of the genus *Leishmania*. Three different forms of the disease are described: visceral, cutaneous and muco-cutaneous leishmaniasis. The disease is endemic in 89 countries, leading to 500,000 new cases and 50,000 annual deaths, mostly due to the visceral form caused by *Leishmania donovani*.[4]

In most of the developing countries the therapy is still based on pentavalent antimonials as first choice drug, whereas amphotericin B, miltefosine, paromomycine and pentamidine are considered second-line drugs. However, all these drugs may cause several side effects, are expensive and toxic; the treatment is prolonged, and antimonial-resistant parasites have emerged in several endemic areas.[5]

Clofazimine (Fig. 1) is a fat-soluble riminophenazine dye used in combination with rifampicin and dapsone as multidrug therapy (MDT) for the treatment of leprosy. It is included in the World Health Organization's List of Essential Medicines, a list of the most important medications needed in any basic health system.[6] Recently, the WHO guidelines included clofazimine within a group of 5 drugs for the treatment of Multidrug Resistant Tuberculosis (MDR-TB), especially of cases that are extensively drug-resistant (XDR-TB).[7]

Consequently, several analogs of clofazimine with reduced lipophilicity, obtained through the introduction of a basic moiety in the structure, have been recently synthesized and studied as MDR-TB agents,[8–10] achieving encouraging results. Clofazimine is also described to possess antiprotozoal activity, exhibiting a moderate antimalarial activity in murine models[11] and antileishmanial

* Corresponding author. Tel.: +39 02 50319365; fax: +39 02 50319359.
E-mail address: anna.sparatore@unimi.it (A. Sparatore).

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effects both in vitro and in vivo;[12,13] however, only few studies are available on this topic. Moreover, tetramethylpiperidine-substituted phenazines (Fig. 1), structurally related to clofazimine, have been described[14] to be endowed with activity against multidrug resistant strains of *P.f.*

In the search for more effective alternatives to the presently used antileishmanial drugs and with the aim to study more thoroughly the antimalarial potentialities of this kind of structures, we synthesized and tested a set of novel riminophenazines bearing a bicyclic basic head, as a quinolizidine or a pyrrolizidine, linked through an alkyl chain to the imino nitrogen in position 3 on the phenazine nucleus (Fig. 2, series A). The choice of this kind of basic heads is related to our previous successful experience with 1-quinolizidinylmethyl and 7-pyrrolizidinymethyl derivatives of 7-chloro-4-aminoquinoline, named AM-1 and MG-3, respectively (Fig. 3), which exhibited an excellent in vitro activity against chloroquine-sensitive (CQ-S) and chloroquine resistant (CQ-S) strains of *P. falciparum* and good in vivo efficacy in murine models of malaria.[15–18] In addition to the riminophenazine derivatives bearing a ω-(quinolizidin-1α/1β-yl)alkylamino or a pyrrolizidinylethylamino group on position 3, which have been previously prepared and studied by some of us for antitubercular activity,[19] we have now synthesized new achiral quinolizidine derivatives connected to the imino group in position 3 of the phenazine through an alkyl chain attached to the bridgehead carbon 9a, thus avoiding any stereochemical issue. To evaluate the role of the steric hindrance of the bicyclic basic heads, the smaller pyrrolidinyl derivative **5** was also studied.

To complete the study and to evaluate the contribution of the increment of polarity on the antiprotozoal activity, we also prepared compounds characterized by the replacement of the aniline moiety in position 2 with an aminopyridine (Fig. 2, series B) or by the quaternarization of the basic nitrogen in the side chain with a methyl group (compound **17**).

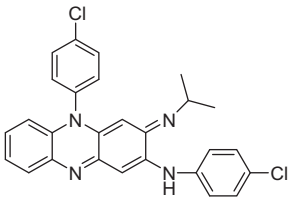
2. Results and discussion

2.1. Chemistry

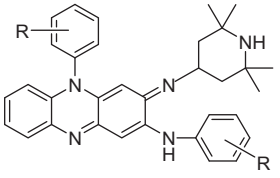
Compounds of Figure 2 were synthesized by reacting, in dioxane solution, the 2-[(4-R-phenyl)amino]-10-(4-R-phenyl)-2,10-dihydro-3-iminophenazines hydrochloride (**22**–**26**) with the suitable cyclic alkylamines, ((1*S*,9a*R*)- or (1*R*,9a*R*)-(octahydro-2*H*-quinolizin-1yl)alkylamines, (hexahydro-1*H*-pyrrolizin-1-yl)alkylamines, (octahydro-1H-quinolizin-9a-yl)alkan-1-amines and 3-(pyrrolidin-1-yl)propylamine in analogy to the previously described riminophenazines[8,20] (Scheme 1).

The quaternary ammonium salt **17** was obtained by reacting compound **12** with iodomethane, as shown in Scheme 2.

The required iminophenazines hydrochloride **22**–**26** were prepared by oxidation of the corresponding N^1-(substituted-phenyl)benzene-1,2-diamine (in acid solution) with ferric chloride.[21]



Clofazimine



TMP-substituted phenazines

Figure 1. Structures of clofazimine and tetramethylpiperidine-substituted phenazines.

As reported by Zhang et al.[9] and by Liu B. et al.[22] phenylpyridinyliminophenazine compounds (**27**–**29**) have been prepared in five steps, as indicated in Scheme 3. The suitable substituted N^1-phenylbenzene-1,2-diamines, previously synthesized or commercially available, were condensed with 1,5-difluoro-2,4-dinitrobenzene in the presence of triethylamine (TEA). Nitrocompounds (**30** and **31**) were reacted with pyridin-3-amine or 6-methoxypyridin-3-amine to afford compounds **32**–**34,** which after reduction with metallic zinc and acetic acid to the corresponding diamino derivatives, underwent a spontaneous oxidative cyclization reaction. The obtained imino compounds (**27**–**29)** were then reacted with 4-(octahydro-1*H*-quinolizin-9a-yl)butan-1-amine or with N^1,N^1-dimethylpropane-1,3-diamine to afford the final products (**18**–**21**).

The required ω-(octahydro-2*H*-quinolizin-1-yl)alkanamines were prepared as previously described by some of us[23–25] and the (hexahydro-1*H*-pyrrolizin-7a-yl)methanamine and 2-(hexahydro-1*H*-pyrrolizin-1-yl)ethylamine have been obtained as described by Miyano et al.[26,27]

Finally, ω-(octahydro-1*H*-quinolizin-9a-yl)alkan-1-amines were obtained in six steps, as indicated in Scheme 4. δ-Valerolactam was reacted firstly with sodium hydride and then with ethyl 5-bromovalerate to generate the corresponding tertiary amide **35**. After treatment with soda lime and distillation, the obtained 2,3,4,6,7,8-hexahydro-1*H*-quinolizine was converted into the corresponding perchlorate **36**.[28,29] Compound **36** was treated with the proper Grignard reagent to generate the olefinic quinolizidines (**37** and **38**),[29,30] which underwent a hydroboration reaction to obtain the alcoholic derivatives (**39** and **40**).[29] After a Mitsunobu reaction in the presence of phthalimide, DEAD and triphenylphosphine and the following acidic hydrolysis,[31] the desired (octahydro-1*H*-quinolizin-9a-yl)alkan-1-amine dihydrochloride salts (**43** and **44**) were obtained.

2.2. Biological activity

The 21 compounds of Figure 2 were tested in vitro against D10 (CQ-S) and W2 (CQ-R) strains of *P. falciparum*. Their antiplasmodial activity was quantified as inhibition of parasite growth, measured as the activity of parasite lactate dehydrogenase (pLDH)[32] and the results, expressed as $IC_{50} \pm SD$ (μM), are reported in Table 1. The ratios between the IC_{50} of each compound against the CQ-R and CQ-S strains of *P. falciparum* are also indicated. This value (resistance index, R.I.) is suggestive of cross resistance between the compound and chloroquine. Cytotoxicity on a human endothelial cell line (HMEC-1) was assayed using the MTT test[33] and the selectivity index (S.I.; IC_{50} HMEC-1/IC_{50} on different strains of *P. falciparum*) was calculated (Table 1).

Seventeen of these compounds were also tested in vitro against different species of *Leishmania* promastigotes, using the MTT assay[34,35] and the results, expressed as $IC_{50} \pm SD$ (μM), are reported in Table 1 together with their corresponding selectivity indexes (IC_{50} HMEC-1/IC_{50} on different species of *Leishmania* promastigotes).

All the tested compounds inhibited the growth of different species of *Leishmania* promastigotes as well as CQ-S and CQ-R strains of *P.f.* Most of them exhibited an IC_{50} in the nanomolar range, with a clear improvement of potency compared to clofazimine, which was, generally, from one to two orders of magnitude less potent. These results confirm the importance of the introduction of a basic head on the imino nitrogen in position 3 on the phenazine.

Concerning the antiplasmodial activity, the tested compounds exhibited similar activity against CQ-S and CQ-R strains of *P.f.*, with very low R.I. (0.4–2.4), thus demonstrating that this kind of compounds do not share the same resistance mechanisms of CQ.

The most active compound on both *P.f.* strains was the quinolizidinylpropyl derivative **9**, which inhibited the D10 (CQ-S) and W2