ANTIPROTOZOAL ACTIVITY OF NOVEL DIARYLIMINOPHENAZINES

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Introduction

Recently, we synthesized a set of novel iminofenazines bearing a bicyclic basic head linked through an alkyl chain to the imino nitrogen in position 3 on the phenazine nucleus (Fig.1). Most of these compounds inhibited the growth of different species of *Leishmania* promastigotes as well as of chloroquine sensitive (CQ-S) and chloroquine resistant (CQ-R) strains of *P. falciparum* with IC_{50} in the submicromolar range (i.e. compound GM05). Unfortunately, these compounds exhibited also a significant toxicity against the human endothelial cell line HMEC-1 with IC_{50} in the low micromolar range and with a consequent low selectivity index (range 2-6).[1] To continue the studies on the antiprotozoal potentialities of this class of compounds and with the aim to improve their activity and selectivity on protozoa, we have now synthesized novel compounds characterized by the replacement of the aniline moiety in position 2 of the phenazine nucleus with an aminopyridine,[2] and/or by a quaternarization of the basic nitrogen in the side chain with a methyl group (Fig. 2).

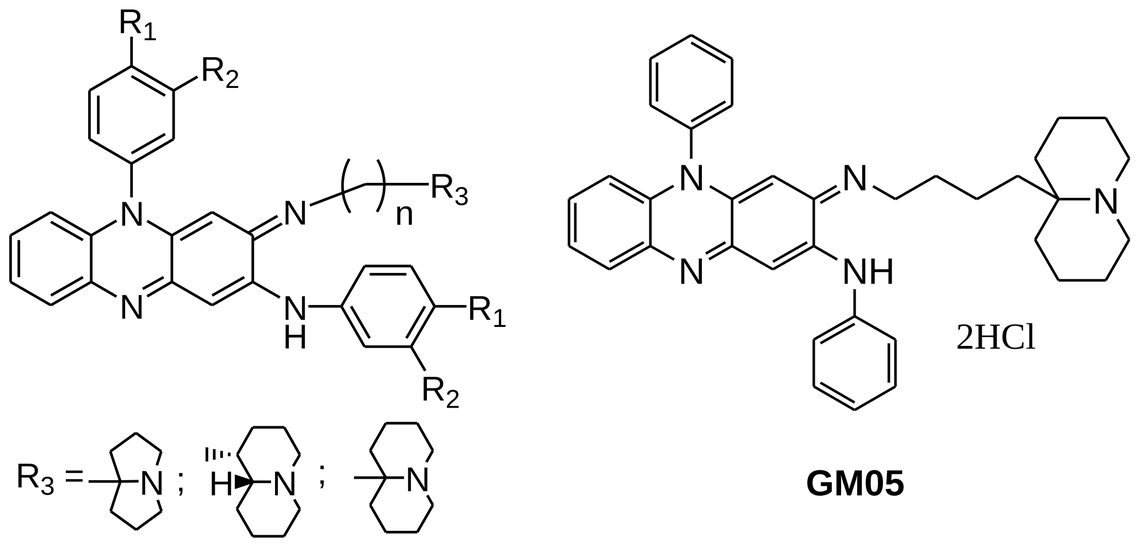


Fig. 1. Structures of the previously synthesized compounds.

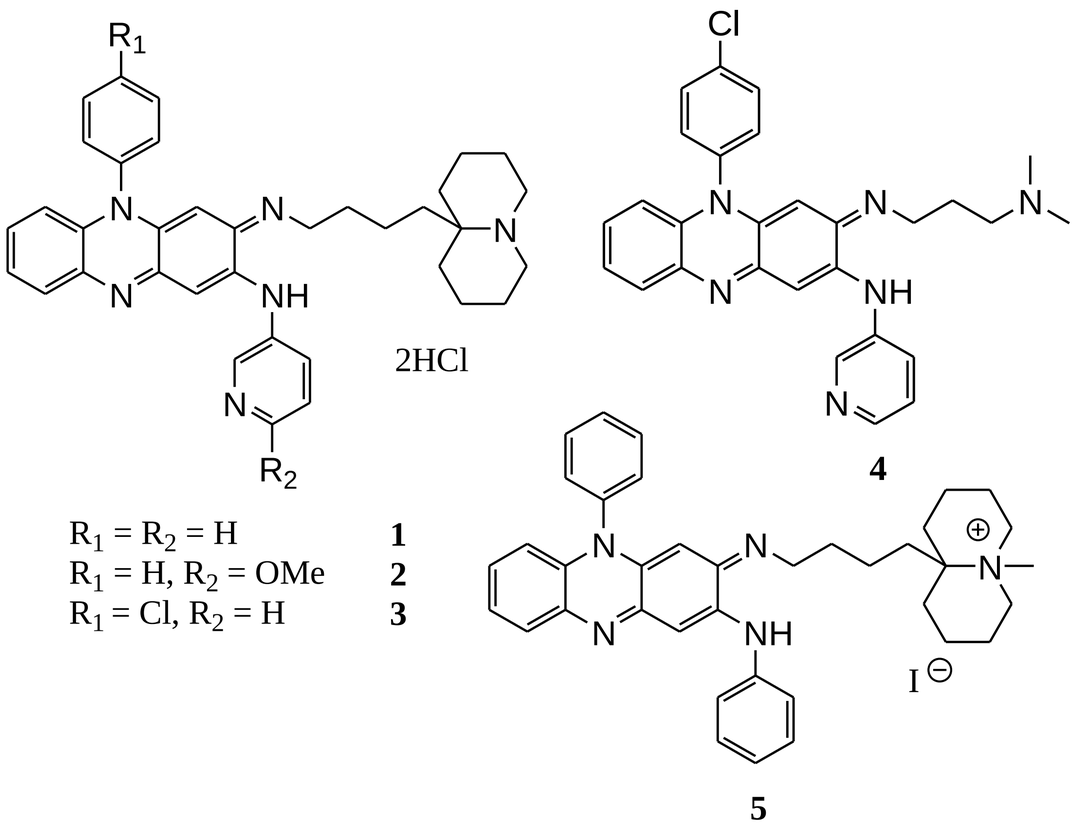
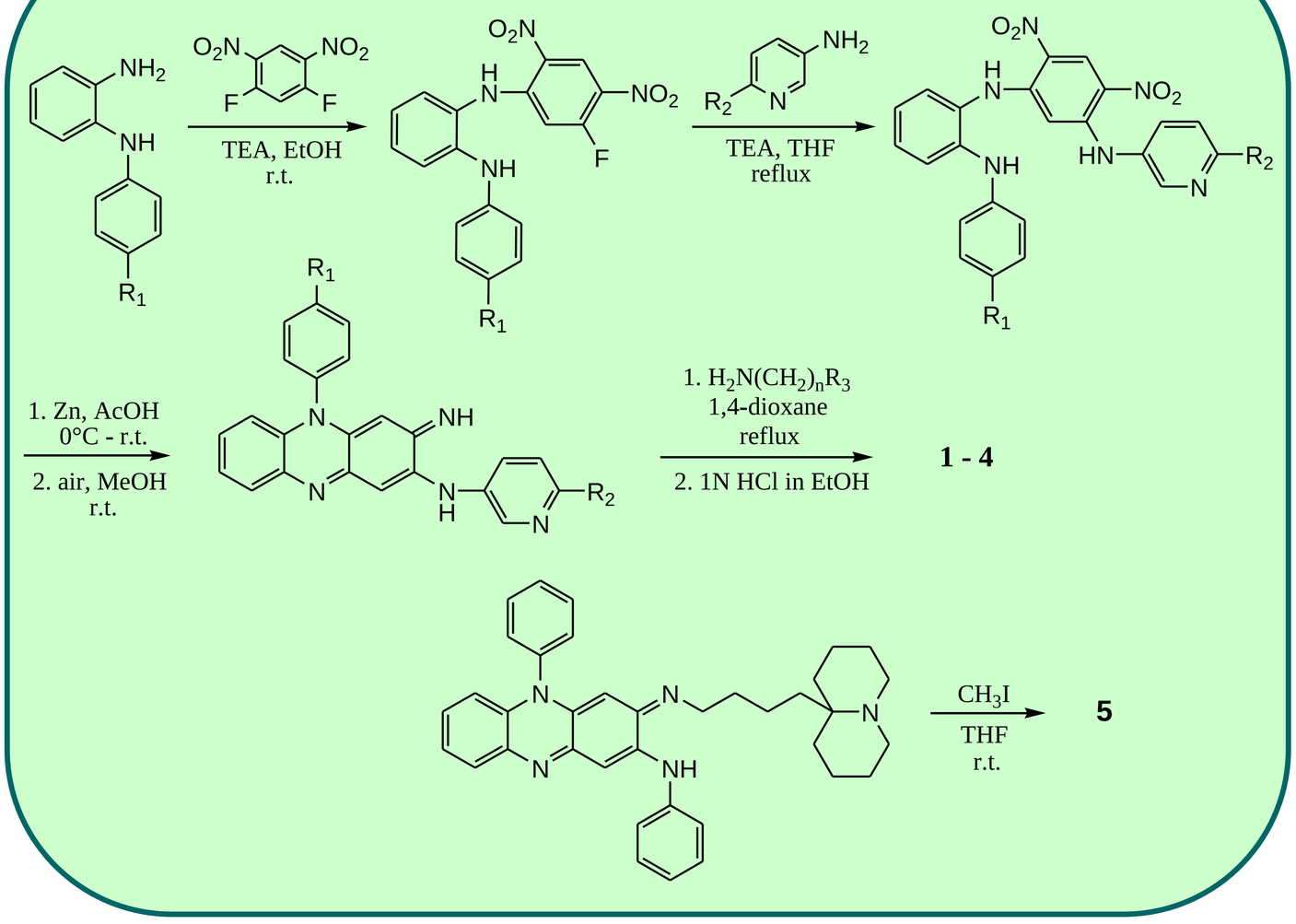


Fig. 2. Structures of the new compounds.

Synthetic Route



Methods

The antimalarial activity was quantified as inhibition of *P. falciparum* growth, measured with the parasite lactate dehydrogenase activity of D-10 (CQ-S) and W-2 (CQ-R) strains. The antileishmanial activity and the cytotoxicity on the mammalian cell line (HMEC-1) was tested using the MTT assay, after 72h of incubation.
Results (Table 1) are expressed as IC_{50}±SD of at least three different experiments, each performed in duplicate.

Results and Conclusions

- **All the new compounds inhibited the growth of CQ-S and CQ-R strains of *P. falciparum* with IC_{50} in the nM range, with a clear improvement of the selectivity index (S.I.), compared to those of GM05 and other compounds of the previous series.**
- **Interestingly, the introduction of the aminopyridine in position 2 of the phenazine nucleus led to higher activity against the CQ-R strain of *P. falciparum* compared to the CQ-S strain, suggesting that this kind of modification could reduce the development of the resistance mechanism.**
- **The replacement of the aniline moiety in position 2 with an aminopyridine moiety or the quaternarization of the quinolizidine nitrogen were not useful for the antileishmanial activity. However, compounds 3 and 4, characterized by the presence of a chlorine in the *para* position of the phenyl ring, maintain good activity against *Leishmania* promastigotes, with IC_{50} in the nM range.**
- **The quaternarization of the basic nitrogen on the side chain seems useful to improve the selectivity index, mainly versus *P. falciparum.***

Table 1. *In vitro* activity against two different species of *Leishmania* promastigotes, antimalarial acitvity against D-10 and W-2 strains of *P. falciparum* and cytotoxicity on the human endothelial cell line (HMEC-1).

Comp.	*L. infantum* IC_{50} (μM)	S.I.	*L. tropica* IC_{50} (μM)	S.I.	D-10 (CQ-S) IC_{50} (μM)	S.I.	W-2 (CQ-R) IC_{50} (μM)	S.I.	HMEC-1 IC_{50} (μM)
1	6.02 ± 2.70	0.6	2.01 ± 0.51	1.9	0.28 ± 0.05	14	0.21 ± 0.06	18	3.89 ± 0.73
2	4.44 ± 0.21	1.4	2.32 ±0.24	2.7	0.81 ± 0.25	8	0.35 ± 0.12	18	6.32 ± 1.64
3	0.34 ±0.07	7	0.34 ± 0.11	7	0.22 ± 0.03	11	0.18 ± 0.05	13	2.33 ± 1.03
4	0.41 ±0.21	11	0.77 ±0.31	6	0.40 ± 0.03	12	0.28 ± 0.04	16	4.66 ± 1.61
5	2.67 ± 1.53	4	1.09 ±0.34	10	0.19 ± 0.03	58	0.27 ± 0.03	41	11.06 ± 3.7
GM05	0.37 ± 0.11	3.4	0.22 ± 0.08	6	0.21 ± 0.03	6	0.27 ± 0.03	4.5	1.23 ± 0.03
AMPH B	0.08 ± 0.02	321	0.09 ± 0.04	285	n.t.		n.t.		25.7 ± 1.90
CQ	n.t.		n.t.		0.02 ± 0.005	>1900	0.32 ± 0.05	>119	>38

n.t. = not tested.

References: 1) Barteselli, A. *et al.,* XXII National Meeting on Medicinal Chemistry, Rome 2013.
2) Zhang, D. *et al., J. Med. Chem.*, 2012, 55, 8409-17.