DESIGN, SYNTHESIS AND STUDY OF RELATIONSHIP STRUCTURE ANTIPROTOZOA ACTIVITIES OF SOME 5-CHLOROBENZIMIDAZOLYL-CHALCONES

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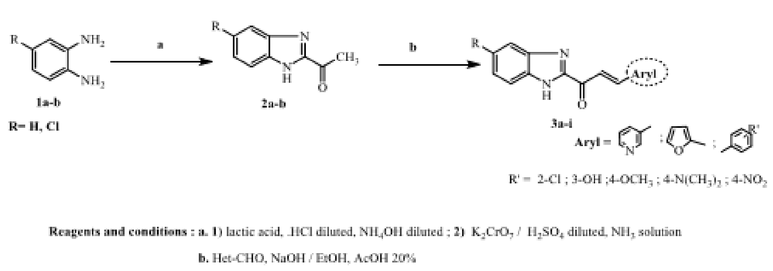
Introduction

Leishmaniasis, trypanosomiasis and malaria are considered as a serious health problem worldwide, especially in Africa where they have significant morbidity and mortality. Faced with the resurgence of drug resistance to antiprotozoa and the paucity of the antiparasitic arsenal against neglected tropical diseases, the search for more effective drugs is necessary. Therefore, it is important to orient pharmacological researches on antileishmanial drugs which exhibit selective efficacy and tolerable safety. For our research, we are interested in benzimidazole, since it constitutes a starting point for the development of new antiprotozoal agents since this nucleus exists in several pharmacologically significant molecules, in particular antiparasitic properties. The present study aimed to identify a molecular hit with a 5-chlorobenzimidazole chalcone profil, which could be developed as an antiprotozoal drug candidate.

Material and methods

1. Chemistry

1 Synthesis of benzimidazolyl-chalcones (**3a-c**)



Reagents and conditions : a. 1) lactic acid, HCl diluted, NH_4OH diluted ; **2)** K_2CrO_7 / H_2SO_4 diluted, NH_3 solution
b. Het-CHO, NaOH / EtOH, AcOH 20%

Scheme 1. General synthesis method of 5-chlorobenzimidazolylchalcones

2. BIOLOGICAL STUDY

2.1. Leishmanicidal activities *in vitro*

The antileishmanial activity of the synthesized compounds was evaluated *in vitro* against *L. donovani* LV9 (MHOM/ET/1967/L82) strain, according to a method basedon a dye.

The screening was performed in flat-bottomed 96-wellplastic tissue-culture plates maintained at 27 °C. The evaluation of anti-leishmanial activity, expressed as an EC_{50}, was carried out by a direct reading under an optical microscope and by Microculture Tetrazolium Assay (MTA). The results are expressed as the effective concentrations inhibiting parasite growth by 50% (EC_{50}) after a 3-day incubation period. The pentamidine was used as reference compound.

2. 2 Antiplasmodial screening method

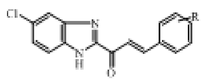
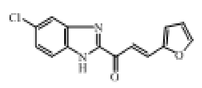
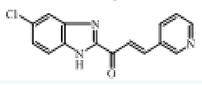
The antiplasmodial activity of these benzimidazolylchalcones was carried out according to the Rieckmann microtest technique. An *in vitro* histidine-rich protein 2 (HRP2) assay was performed to assess anti-*P. falciparum* activity. HRP2 assays were performed according to standard procedures. Absorbance values were read using an ELISA plate reader (iMarkTM, Bio-Rad, USA) at a maximum absorbance of 450 nm. The measurement of antimalarial activity of the drugs was expressed as 50% inhibitory concentration (IC_{50}) Chloroquine was used as a reference molecule with a sensitivity threshold set at 100 µM.

2.3. *In vitro* trypanocidal activity

The parasites were distributed in a 96-well plate of 200 µL at a rate of 2,105 per mL. Then, 5 µL of the appropriate dilution of the compounds in DMSO are added, each concentration being tested in triplicate. The test results were expressed as a lethal concentration killing all the parasites in the wells after 24 hours (LC100, Lethal Concentration) after microscope observation. Melarsoprol was used as reference drug.

Results

1. Antileishmanial activities against *Leishmania donovani*

Structure	Compounds	R	EC_{50} (µM)
	**4a**	H	0.53
	4b	3-OH	0.50
	4c	2-Cl	0.47
	4d	3-NO_2	74.45
	4e	4-CH_3	1.04
	4f	4-Cl	24.59
	4g	2.4-diCl	1.79
	**4h**		1.14
	**4i**		13.79
Pentamidine			7.60

- Based on their activities, the chlorine derivatives of the series can be classified as follows 2-Chloro > 2.4-dichloro > 4-Chloro, with EC_{50} of 0.47 ; 1.79 and 24.59 µM, respectively.
- 3-nitro analogue **4d** was found to be the least active among the 5-chlorobenzimidazole series (EC_{50} = 74.45 µM).
- This observation suggests that the presence of an electron-withdrawing group such as nitro does not allow efficient binding with the target on *Leishmania donovani*.
- The replacement of the benzene nucleus of phenylpropenone chain with its pyridine-type isostere (compound **4l)** or its furyl-type isostere (compound **4h)** didn't improve their activitie.
- In this series, seven of the tested compounds (**4a-c, 4e,4g**, **4h**, **4j**) exhibited remarkable inhibitory activity with EC_{50} ranging from 0.5 to 1.8 µM. These compounds were at least 4-fold more active against *L. donovani* strain than pentamidine (7.6 µM).

SAR studies led to the characterization of a lead compound (ortho chloro derivative **4c**) that showed very good *in vitro* activity against *L. donovani*.

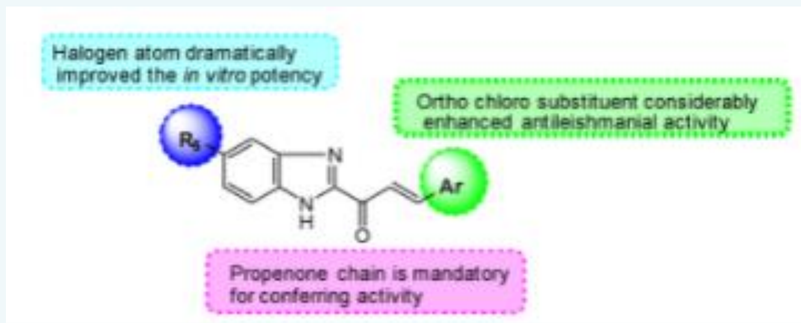


Fig 1 : SAR of benzimidazolyl-chalcone derivatives as antileishmanial agents

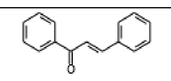
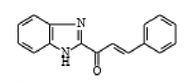
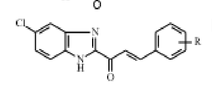
2. Trypanocidal activities against *Trypanosoma brucei brucei*

Table 2. Trypanocidal activity against *Trypanosoma brucei brucei* of selected compounds

Compounds	R	LC_{100} (µM)
4a	H	2
4c	2-Cl	1
4d	3-NO_2	50
Melarsoprol		0.5

- Interestingly, results of trypanocidal activity showed that selected compounds previously active against *L. donovani* (4a and 4c) are also active on trypanosomes.
- Likewise, compound 4d was found to be moderately active against Leishmania donovani as well as on *Trypanosoma brucei brucei*, with a LC_{100} value of 50µM.

3. Antiplasmodial activities

Compounds	Structures			Chloroquine-sensitive *P. falciparum* isolate IC_{50} (µM) ±SD	Chloroquine-resistant *P. falciparum* isolate IC_{50} (µM) ± SD
Chalcone or 1,3-diphenyl prop-2-en-1-one				38.56 ± 3.86	1.44 ± 0.14
3a[14]				44.38	6.81
3b		R	H	10.65 ± 1.07	0.78 ± 0.08
3c			2-Cl	32.10 ± 3.21	31.28 ± 3.13
3d			3-OH	6.23 ± 0.62	2.34 ± 0.23
3e			4-OCH_3	0.32 ± 0.03	1.96 ± 0.20
3f			4-$N(CH_3)_2$	9.40 ± 0.94	1.82 ± 0.18
3g			4-NO_2	29.39 ± 2.94	10.61 ± 0.11

Overall, general trends of SAR established that

- the replacement of the benzene homocycle by a heterocycle is not appropriate to have excellent antiplasmodial activities.
- the electro withdrawing groups seems to induce a reduction of activity on chloroquinosensitive isolates as well as on chloroquine-resistant isolates
- - the presence of methoxygroup, a modulator of the antiplasmodial activity of quinine, increased the antiplasmodial efficacy only on chloroquinosensitive isolates.

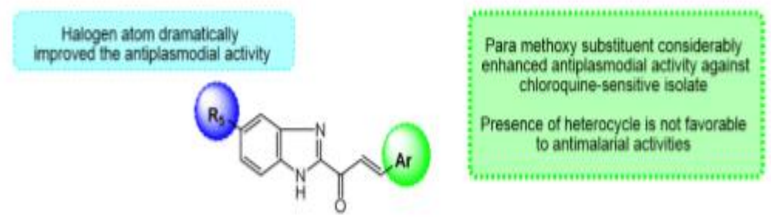


Fig. 3. SAR of benzimidazolyl-chalcones with antiplasmodial properties

Conclusion

General investigation of SAR established that chalcone hybrids of 5-chlorobenzimidazole furnished in general highly active compounds against *all tested protozoa*. The SAR studies show that:
- The presence of halogen and the propenone chain at C5 and C2 of benzimidazole, respectively, increases the in-vitro antiprotozoal potentialities
- Substitution of phenylpropenone benzene does not improve the antiprotozoal activities
- Non-substitution of phenyl exudes antiplasmodial activities on CQR strains
- Introduction of a chlorine in ortho of the benzene homocycle improves antileishmanial and antitrypanosomal activities

The 5-chlorobenzymidazole motif carried by the phenylpropenone moiety can be considered as an antiprotozoal pharmacophore including antiplasmodial, antileishmanial and trypanosomicide

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