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# *In vitro* anti-leishmanial and anti-trypanosomal activity of hydrazones, pyrazoles, pyrazolo[1,5-*a*]pyrimidines and pyrazolo[3,4-*b*]pyridine, synthesized from 6-substituted-3-formylchromones

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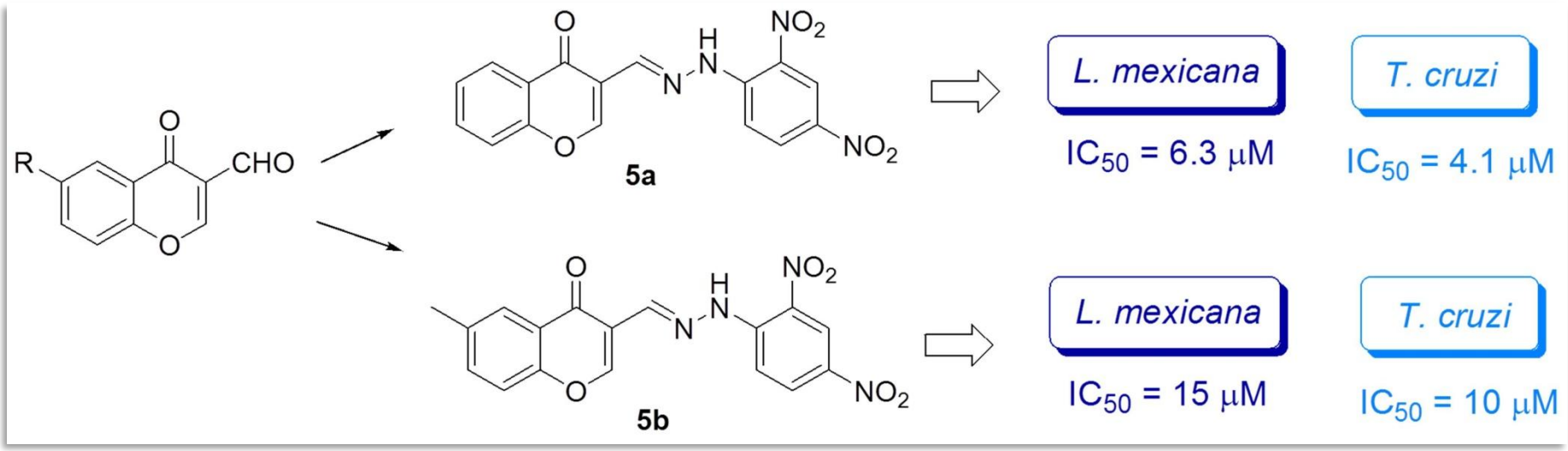
# *In vitro* anti-leishmanial and anti-trypanosomal activity of hydrazones, pyrazoles, pyrazolo[1,5-*a*]pyrimidines and pyrazolo[3,4-*b*]pyridine, synthesized from 6-substituted-3-formylchromones

## Graphical Abstract



## Abstract

Led by the biological and pharmacological relevance of the 3-formylchromone derivatives and its interesting chemistry, in this work we present the synthesis of a series of pyrazoles (**4a-c**), hydrazones (**5a-c**), pyrazolo[1,5-*a*]pyrimidines (**6a**, **6b**) and one pyrazolo[3,4-*b*]pyridine (**7**) and the report on their *in vitro* anti-leishmanial and anti-trypanosomal activity. Chemical results showed that the formation of regioisomer **7** may arise from an imine intermediary that undergoes 1,4-addition by attack of C-4' from the pyrazole. To the best of our knowledge, this is the first report regarding formation of pyrazolo[3,4-*b*]-pyridines by intramolecular attack of an $sp^2$ carbon atom.

The *in vitro* studies were performed against strains of *Leishmania mexicana* (bel 21) and *Tripanosoma cruzi* (DM28). Compounds **5a** and **5b** showed activity at micromolar level and good selectivity index (SI) with $IC_{50}$ values of 6.3 (SI = 3.4) and 15 (SI = 1.9) mM for *L. Mexicana* and 4.1 (SI = 5.2) and 10 (SI = 3) mM for *T. cruzi* respectively. From the above-mentioned, compounds **5a** and **5b** may be considered for further chemical modifications in order to increase their activity as potential antiparasitic agents.

**Keywords:** Anti-leishmanial, anti-trypanosomal, 6-substituted-3-formylchromones

## Introduction

The research of compounds with antiparasitic properties is a matter of great importance. Chaga's disease is present mostly in countries of South America with about 5-6 million individuals infected and 25 million at risk [1]. Leishmaniasis on the other hand, is a growing public health problem, with an annual incidence of about 1.3 million cases [2].

Nitrogenated derivatives such as hydrazones, pyrazoles, pyrazolo[1,5-*a*]pyrimidines and pyrazolo[3,4-*b*]pyridines are widely recognized as an important class of biological active substances [3-8]. They have shown several biological and pharmacological activities, including cytotoxic, antitrichomonal, antischistosomal, antichagasic and antimalarial effects. The presence of these nitrogenated groups is also observed in commercial drugs. Mebendazole [9], a well known antiparasitic compound, presents amino imidazole and methanone moieties while the antileishmaial drug allopurinol [10] bears a pyrazolo[1,5-*a*]-pyrimidine nucleus.

In a previous work, high toxicities of pyrazole **4a-c** hydrazones **5a-c** pyrazolo[1,5-*a*]pyrimidines **6a**, **6b** and pyrazolo [3,4-*b*]pyridine **7** compounds were observed against *Artemia salina* through the brine shrimp lethality assay (BSLA) [11]. This anti-larvae property may be indicative of trypanocidal action [12]. In this study we decided to evaluate the *in vitro* antileishmanial and antitrypanosomal activity of those nitrogenated derivatives, against strains of *L. mexicana* and *T. cruzi.*

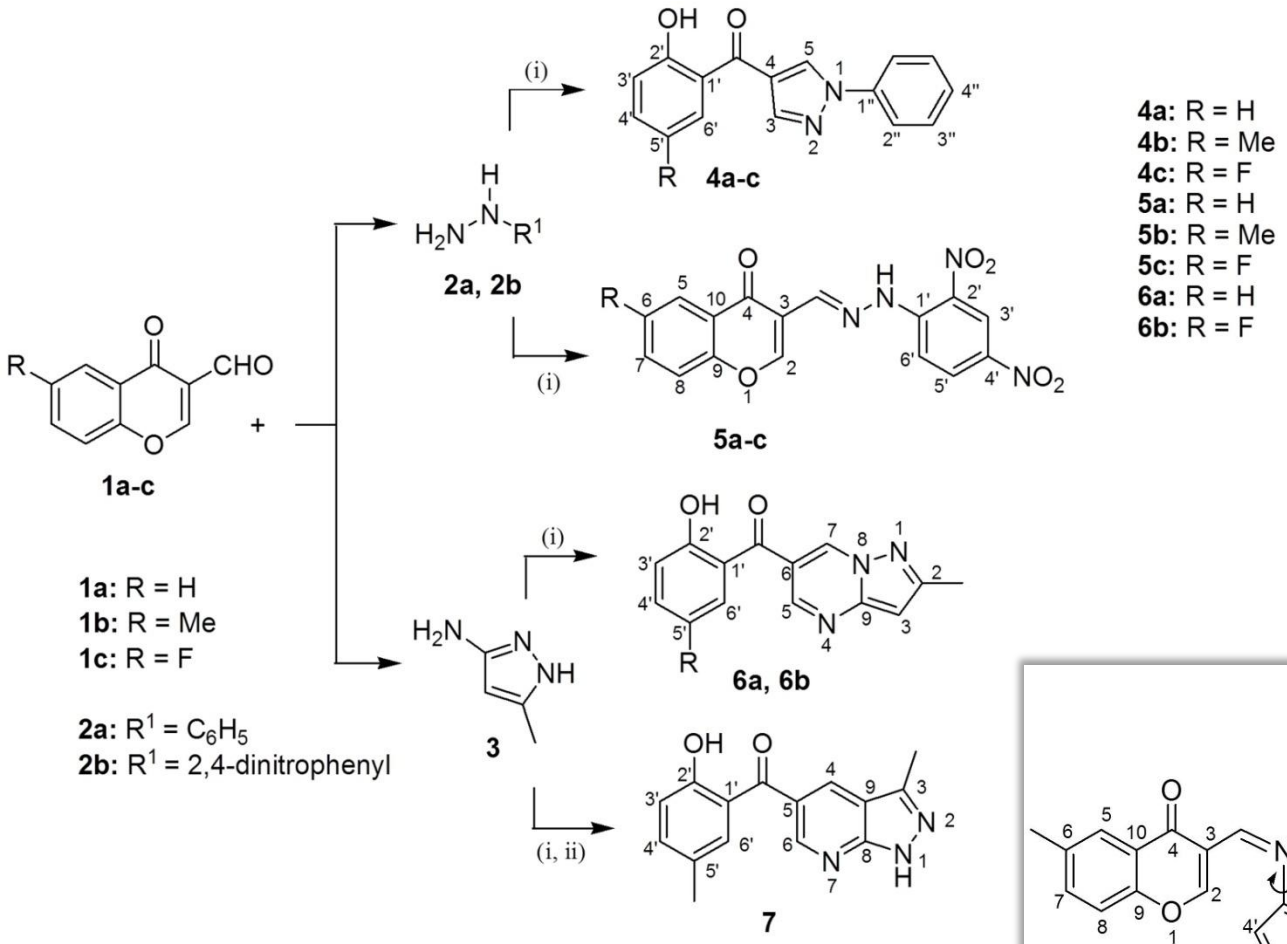
## Results and discussion

### Synthesis

The products **4a-c**, **5a-c**, **6a** and **6b** were obtained from the reaction between equimolar quantities of 3-formylchromone derivatives **1a-c**, with the corresponding hydrazines **2a**, **2b** or aromatic amine **3** in anhydrous THF **(Scheme 1)**.

The pyrazolo[3,4-*b*]pyridine **7** was obtained in two steps: the reaction of **1b** with **3** produced a precipitate which was then refluxed using AcOH as solvent in presence of $I_2$ **(Scheme 1)**. The formation of regioisomer **8** may arise from an imine intermediary **(Scheme 2)** that undergoes 1,4-addition at C-2 by attack of C-4' from the pyrazole instead of the nitrogen atom N-2'. To the best of our knowledge, this is the first report regarding formation of pyrazolo[3,4-*b*]-pyridines by intramolecular attack of an $sp^2$ carbon atom.

All reactions were monitored by TLC and obtained in good yields (80–95%). The compounds were fully characterized using NMR, IR and MS methods; all physical constants and spectroscopic data were in accordance with those reported in the literature [11,13,14,19].

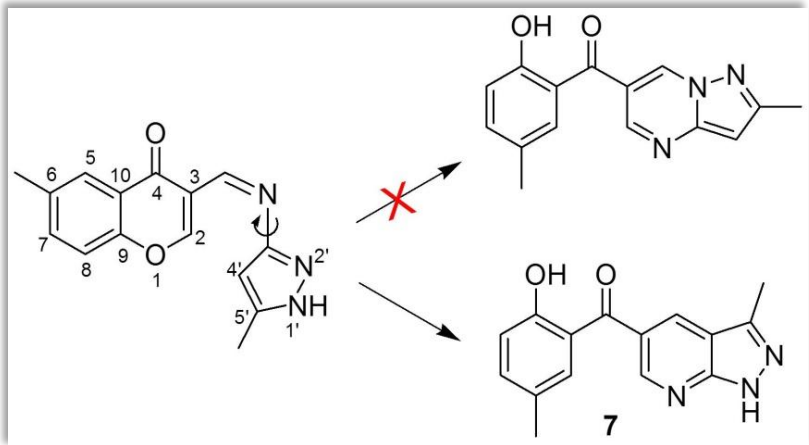



**4a:** R = H
**4b:** R = Me
**4c:** R = F
**5a:** R = H
**5b:** R = Me
**5c:** R = F
**6a:** R = H
**6b:** R = F

**1a:** R = H
**1b:** R = Me
**1c:** R = F

**2a:** $R^1 = C_6H_5$
**2b:** $R^1$ = 2,4-dinitrophenyl

**Scheme 1.** Reagents and conditions: (i) THF, reflux (2h); (ii) AcOH/$I_2$, reflux (4h)




**Scheme 2.** Formation of pyrazolo[3,4-*b*]pyridine **7**

## Antileishmanial and anti-trypanosomal activity

The nitrogenated compounds **4a-c**, **5a-c**, **6a**, **6b** and **7** were tested for leishmanicidal and trypanocidal activities. Primary cultures of human dermal fibroblast were used as reference. The selectivity action of the compound over the parasite was calculated using the selectivity index (SI), which is expressed as the ratio of $IC_{50}$ on fibroblast to the $IC_{50}$ of the corresponding parasite strain.

As observed in **Table 1**, all tested compound showed good to moderate activity. Compound **5a** exhibited the strongest antiparasitic activity against both parasites *L. mexicana* and *T. cruzi* with $IC_{50}$ values of 6.3 ± 0.7 μM and 4.1 ± 0.3 μM respectively. Compound **5a** also displayed the highest SI with values of 3.4 for *L. mexicana* and 5.2 for *T. cruzi*. Therefore, **5a** was the most effective compound tested against both parasites. Compounds **5b** and **5c** also exhibited good antiparasitic activity since their $IC_{50}$ values were relatively low. When **5b** was tested against *L. Mexicana*, the $IC_{50}$ was 15 ± 3 μM having a SI value of 1.9, while against *T. cruzi* the $IC_{50}$ value was 10 ± 1 μM with a SI value of 3. **5c** showed activity only for *T.cruzi* ($IC_{50}$ = 13 ± 1 μM) with SI value of 2. Compounds **4b** and **7** exhibited moderate activity against *L. mexicana* ($IC_{50}$ = 25 ± 2 and 35 ± 2 μM, respectively) but in the case of compound **7** the selectivity was poor.

We compared the antiparasitic results action of **4a-c**, **5a-c**, **6a-6b** and **7** with their antilarvae properties [11], and found that the most active compounds against *L. mexicana* and *T. cruzi* were also among the most toxic to *A. salina*. We also evaluated the correlation between leishmanicidal and trypanocidal activities of the synthesized compounds, using a linear regression method. There was a significant association ($p<0.05$) between both antiparasitic effects. The correlation coefficient was 0.71, indicating a moderately strong relationship. So the most active compounds could be evaluated in a wider range of parasite strains.

The strongest antiparasitic activity was found in the series **5a-c**, which has a hydrazone-type structure bearing a 2,4-dinitrophenyl moiety. The hydrazone nucleus has been found to be important in the leishmanicidal activity [15]. The presence of the nitro groups could also contribute to the activity since they are able to play a role in antiprotozoal substances [16-18].

From the above-mentioned, compounds **5a** and **5b** may be considered for further chemical modifications, in order to increase their activity and selectivity as potential antiparasitic agents.

**Table 1**: *In vitro* leishmanicidal and trypanocidal activities of synthesized compounds against *L. mexicana* and *T. cruzi*.

| Comp. | *L. mexicana* | | *T. cruzi* | | Fibroblasts |
|---|---|---|---|---|---|
| | $IC_{50}$ (μM) | SI | $IC_{50}$ (μM) | SI | $IC_{50}$ (μM) |
| **4a** | 37 ± 4 | 0.3 | 57 ± 9 | <0.7 | 11 ± 1 |
| **4b** | 25 ± 2 | 3.0 | > 90 | <0.8 | 73 ± 11 |
| **4c** | 53 ± 13 | - | 174 ± 60 | - | ND |
| **5a** | 6.3 ± 0.7 | 3.4 | 4.1 ± 0.3 | 5.2 | 22 ± 5 |
| **5b** | 15 ± 3 | 1.9 | 10 ± 1 | 3.0 | 28 ± 7 |
| **5c** | > 23 | 1.3 | 13 ± 1 | 2.2 | 29 ± 4 |
| **6a** | 89 ± 9 | - | 107 ± 25 | - | ND |
| **6b** | 60 ± 3 | - | 93 ± 16 | - | ND |
| **7** | 35 ± 2 | 0.6 | 35 ± 4 | 0.6 | 19.5 ± 0.4 |

**$IC_{50}$:** concentration of the compound that induces 50% growth inhibition in 48 h. Values are expressed as the mean ± SD; SI: selectivity index, is defined as the ratio of $IC_{50}$ on fibroblasts to the $IC_{50}$ on the corresponding parasite; ND: not determinated.

## Materials & Methods

### *General procedure for the synthesis of* 4a-c *and* 5a-c

3-Formylchromone derivative **1a-c** (1.0 mmol) was dissolved in hot anhydrous THF (4 mL) and a solution of hydrazines **2a**, **2b** (1.0 mmol) in THF (2 mL) was added slowly. The mixture was refluxed for 1–2 h, and once cooled the solid was filtered, washed with water and recrystallized from absolute EtOH.

### *General procedure for the synthesis of* 6a *and* 6b

3-Formylchromone derivative **1a** or **1c** (1.0 mmol) was mixed with aminopyrazole **3** (1.0 mmol) and refluxed in 10 mL of anhydrous THF for 1 h. After cooling, the solid was filtered, washed repeatedly with hot THF and recrystallized from EtOH to give TLC pure compounds.

### *General procedure for the synthesis of* 7

Equimolar quantities of **1b** and **3** (1.0 mmol) were refluxed in anhydrous THF for 1-2 h, until the formation of a precipitate. Once separated from the solution, this precipitate was dissolved in AcOH (7 mL) in presence of $I_2$ (1.0 mmol) and refluxed for a period of 3-4 h. After completion of the reaction by TLC, the mixture was poured into crushed ice and treated with $NaHCO_3$ and $Na_2SO_3$. The solid was filtered, washed with cold water and dried.

## Antileishmanial and antitrypanosomal activity

Epimastigotes of *T. cruzi* and promastigotes of *L. mexicana* were grown at 26°C in liver infusion tryptose (LIT) medium with penicillin (100 U/mL), streptomycin (100 µg/mL), and 10% heat-inactivated FBS. A modified MTT assay was used to determine the compounds $IC_{50}$ on each parasite strain [20]. Briefly, 199 µL per well of exponential growing parasites at a dilution of $2\times10^6$ cells per mL, were trasfered into a 96-well flat-bottom plate, and compounds or solvents (1 µL compound or DMSO) were added to evaluate different concentrations. Plates were incubated at 26°C for 48h and 5 days for *L. mexicana* promastigotes or *T. cruzi* epimastigotes respectively. Then, parasites were seeded, the medium was removed, and the cells were incubated with MTT solution in PBS (0.2 mg/ml for *L. mexicana* or 0.5 mg/ml for *T. cruzi*), F-formazan crystals were dissolved by addition of 100 µL DMSO after plates were centrifuged and MTT solution was removed. The following equation was used to calculate the percentage of growth inhibition (%GI):

$$\%GI = [1 - (A_p - A_b)/ A_c - A_b)] \times 100$$

$A_p$ is the absorbance of compound-treated cells, $A_c$ is the absorbance of solvent-treated cells, and $A_b$ is the absorbance of culture medium. The $IC_{50}$ value was calculated applying a sigmoidal analysis when %GI and the corresponding concentration were plotted using the Origin 8.0® software (OriginLab Corporation). For each condition evaluated at least three independent assays were performed in triplicate.

## Conclusions

- All synthesized compounds exhibited good to moderate antiparasitic activities against strains of *L. mexicana* and *T. cruzi*.

- Compounds **5a** and **5b** showed the strongest activities with good selectivity indexes. The results suggest that these molecules are suitable candidates for further chemical modification and evaluation as potential anti-leishmanial and anti-trypanosomal agents.

- The good correlation exhibited for the results between both parasite lines indicates that the most active compounds **5a** and **5b**, could be used in a wider range of parasite strains.

- Finally, the comparison of the antiparasitic results with the previous BSLA study allowed us to conclude that this anti-larvae test may be a good and inexpensive tool in predicting trypanocidal action.

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