Synthesis and Biological Evaluation of Benzamide Derivatives Against Chagas Disease and Leishmaniasis

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RESUMO

ABSTRACT-Chagas disease and leishmaniasis, caused by *Trypanosoma cruzi* and *Leishmania infantum* respectively, represent significant public health burdens in tropical regions, affecting millions in Brazil alone. These neglected tropical diseases are transmitted through insect vectors and can lead to chronic infections with severe clinical manifestations. The urgent need for new treatments against Chagas disease and leishmaniasis drives the search for novel therapeutic agents. In this context, we developed compound **1**, a novel synthetic molecule demonstrating potent anti-T. cruzi activity (IC_{50} < 18.14 μM) and modest efficacy against L. infantum (IC_{50} = 106.44 μM). Structural modification yielding analog **8** resulted in significantly reduced activity (*T. cruzi* IC_{50} = 171.02 μM; *L. infantum* IC_{50} = 298.15 μM), revealing critical structure-activity relationships. These findings position compound **1** as a promising lead for developing improved anti-kinetoplastid therapies, particularly for Chagas disease.

Palavras-chave: Chagas disease, Visceral leishmaniasis, Organic synthesis, Structure-activity relationship.

Introduction

Chagas disease and leishmaniasis, caused by the kinetoplastid parasites *Trypanosoma cruzi* and *Leishmania infantum*. (e.g., *L. infantum*, *L. donovani*), are neglected tropical diseases (NTDs) endemic in tropical and subtropical regions. Both diseases lead to severe clinical manifestations (chronic cardiac/digestive pathologies for Chagas disease and visceral/cutaneous forms for leishmaniasis, respectively). The pathogens exhibit distinct host interactions and disease outcomes. Current challenges include drug resistance and the need for improved therapies, highlighting the importance of research into novel diagnostic and therapeutic targets[1]. Conventional treatments face challenges: meglumine antimoniate, amphotericin B (leishmaniasis), and benznidazole (Chagas disease) exhibit variable efficacy and adverse effects, with the latter being ineffective in the chronic phase[1]. Thus, novel approaches are urgently needed. The initial hit compound, referred to as **Hit 1** (Figure 1), was selected due to its promising activity against *T. cruzi* and *L. donovani* (IC_{50} = 0.5 and 10 μM, respectively), low cytotoxicity (CC_{50}> 100 μM), and potential for structural modifications[1].

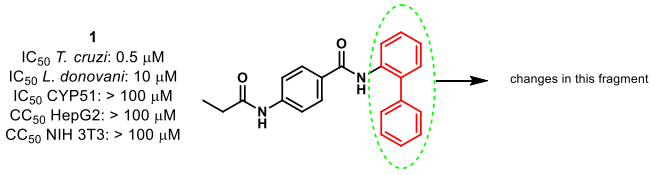


Figure 1. Chemical structure of **1**, potency against *T. cruzi* and *L. donovani*, and cytotoxicity.

This study aims to synthesize and characterize **Hit 1**, confirm its biological activity against *T. cruzi* and *L. infantum*, and investigate the importance of the naphthyl fragment in these biological activities through chemical modifications.

Experimental

Synthetic Route

Compound **3** – 4-Nitrobenzoic acid was esterified in 15mL of anhydrous EtOH with 10 drops of concentrated H_2SO_4 under an Ar atmosphere at 85°C with continuous stirring for 72h, yielding compound **3**.

Compound **4** – The ester (**3**) underwent catalytic hydrogenation (H_2/Pd/C) to reduce the nitro group. The reaction was carried out in 15mL of a (EtOAc1:1MeOH) mixture at room temperature under stirring for 6h. Pd/C was removed by filtration through Celite, and the product was dissolved in MeOH, which was subsequently evaporated for the next step.

Compound **6** – An amidation reaction was performed using compound **4** (1.0 eq) and propanoic acid (compound **5**, 2.0 eq) with EDC (2.0 eq) and HoBt (0.1 eq) in 2mL of DMF at 50°C under stirring for 72h. Distilled water was then added to induce precipitation, followed by extraction with EtOAc. The organic phase was evaporated to afford compound **6**.

Compound **7** – Compound **6** (1.0 eq) underwent alkaline hydrolysis with 2M NaOH (10.0 eq) in 10mL of THF at room temperature under stirring for 17h. The mixture was acidified with concentrated HCl to pH = 1, and the aqueous phase was extracted with EtOAc. The organic phase evaporated to yield compound 7.

Hit 1 – An amidation between compound **7** (1.5 eq) and

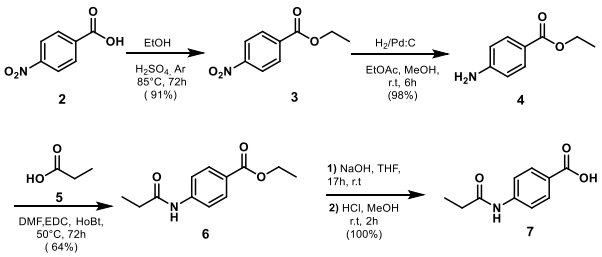
biphenylamine (1.0 eq) was attempted using EDC (2.0 eq) and HoBt (0.1 eq) in 2mL of DMF at 80°C for 144h. After adding distilled water, extraction with EtOAc was performed, but the expected **Hit 1** was not obtained.

Alternative route: Compound **7** (1.0 eq) was converted to its acid chloride using oxalyl chloride (5.0 eq) in 3mL of DCM with 3 drops of DMF as a catalyst under stirring for 3h. Biphenylamine was added, followed by pyridine (5mL), and **Hit 1** was isolated after column chromatography.

Compound **8** – This compound was obtained via amidation of compound 7 (2.0 eq) with aniline (1.0 eq) using EDC (2.0 eq) and HoBt (0.1 eq) in 2mL of DMF at room temperature under stirring for 44h, followed by extraction with EtOAc and solvent evaporation. All reactions were performed under stirring, with EtOAc extractions followed by rotary evaporation. Hazardous materials (flammable Ar/H_2, pyrophoric Pd/C, toxic DMF, irritants EDC•HCl/HOBt, corrosive NaOH/HCl, reactive oxalyl chloride) required fume hood use, PPE (nitrile gloves, goggles, lab coats), and institutional waste protocols.

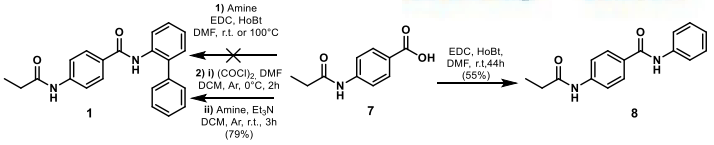
Results and Discussion

The synthetic route to **Hit 1** was successfully developed through a carefully optimized five-step sequence, achieving an overall yield of 45.1%. The process began with a high-yielding esterification step (91% yield) converting 4-nitrobenzoic acid to the corresponding ethyl ester under acidic conditions. This was followed by an exceptionally efficient catalytic hydrogenation (98% yield) using Pd/C under hydrogen atmosphere to reduce the nitro group to an amine. The subsequent amide coupling, while somewhat lower yielding (64%), successfully introduced the key amide functionality. A critical hydrolysis step proceeded quantitatively (100% yield) to generate carboxylic acid intermediate **7**.



Scheme1. Synthesis of intermediate **7**.

The final coupling reaction via acid chloride formation successfully completed the synthesis of **Hit 1** in good yield (79%), demonstrating the robustness of this approach after initial attempts using EDC/HOBt coupling failed. In parallel, we developed an alternative route to access structural analog **8** through direct amidation of the carboxylic acid with aniline using EDC/HoBt (without chlorination), which provided important SAR insightsdespite lower efficiency (31.5% overall yield limited by the 56% yield in the final step). The significant yield difference between routes (45.1% vs 31.5%) highlights both the superiority of the acid chloride method for **Hit 1** and the synthetic challenges encountered with the **8** series.



Scheme 2. Synthesis of **1** and its derivative**8**.

Structure **1** corresponds to **Hit 1**, while structure **8** is the synthesized derivative discussed in this study. The synthetic routes (Schemes 1–2) were designed to systematically implement targeted alterations to probe structure-activity relationships (SAR). Compound **1** exhibited potent anti-*T. cruzi* activity (IC_{50} < 18.14 μM) but limited efficacy against *L. infantum* (IC_{50} = 106.44 μM), while **8** showed no activity against both parasites (*T. cruzi* IC_{50} = 171.02 μM; *L. infantum* IC_{50} = 298.15 μM). The significant 9.4-fold reduction in anti-*T. cruzi* potency observed for analog **8** underscores the detrimental impact of replacing the biphenylamine moiety with aniline. This finding highlights the structural sensitivity of the activity and affirms that the scaffold of compound **1** is well-suited, potentially near-optimal among the analogs tested, for interaction with the relevant kinetoplastid target(s).

Conclusion

The optimized synthesis of **1** (45.1% overall yield) delivered a dual-activity compound showing potent inhibition of *T. cruzi* (IC_{50} <18.14 μM) and moderate activity against *L. infantum* (IC_{50} = 106.44 μM). In contrast, structural modifications yielding **8** (31.5% yield) abolished activity against both parasites (*T. cruzi* IC_{50} = 171.02 μM; *L. infantum* IC_{50} = 298.15 μM). In conclusion, the optimized synthesis yielded **Hit 1**, a compound demonstrating potent activity against *T. cruzi* and moderate activity against *L. infantum*. Structure-activity relationship studies, through the synthesis and evaluation of analog **8**, revealed that the biphenylamine moiety is crucial for maintaining anti-*T. cruzi* potency. These findings validate compound **1** as a promising lead scaffold for the development of improved anti-kinetoplastid agents, particularly for Chagas disease, warranting further optimization studies.

Acknowledgment

CNPq, CAPES, FAPEMIG, LaSFar, LQMC, PPGQUI-UFU, IQUFU.

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