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Synthesis and biological evaluation of potential inhibitors of the cysteine proteases cruzain and rhodesain designed by molecular simplification

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ABSTRACT

Analogues of 8-chloro-*N*-(3-morpholinopropyl)-5*H*-pyrimido[5,4-*b*]indol-4-amine **1**, a known cruzain inhibitor, were synthesized using a molecular simplification strategy. Five series of analogues were obtained: indole, pyrimidine, quinoline, aniline and pyrrole derivatives. The activity of the compounds was evaluated against the enzymes cruzain and rhodesain as well as against *Trypanosoma cruzi* amastigote and trypomastigote forms. The 4-aminoquinoline derivatives showed promising activity against both enzymes, with IC_{50} values ranging from 15 to 125 μM. These derivatives were selective inhibitors for the parasitic proteases, being unable to inhibit mammalian cathepsins B and S. The most active compound against cruzain (compound **5a**; IC_{50} = 15 μM) is considerably more synthetically accessible than **1**, while retaining its ligand efficiency. As observed for the original lead, compound **5a** was shown to be a competitive enzyme inhibitor. In addition, it was also active against *T. cruzi* (IC_{50} = 67.7 μM). Interestingly, the pyrimidine derivative **4b**, although inactive in enzymatic assays, was highly active against *T. cruzi* (IC_{50} = 3.1 μM) with remarkable selectivity index (SI = 128) compared to uninfected fibroblasts. Both **5a** and **4b** exhibit drug-like physicochemical properties and are predicted to have a favorable ADME profile, therefore having great potential as candidates for lead optimization in the search for new drugs to treat Chagas disease.

1. Introduction

Chagas disease, also known as American trypanosomiasis, is an illness caused by the parasite *Trypanosoma cruzi* and transmitted to humans through the bite of infected blood-sucking triatomine bugs. More than 100 years after its discovery, Chagas disease remains neglected and it is still a relevant public health issue, affecting about 6 million people, mostly in Latin America.[1] This disease often occurs in people in the most productive phase of their lives, resulting in huge economic and social impact.[2]

Only two drugs are clinically available for Chagas disease chemotherapy: benznidazole and nifurtimox. Both are options for patients in the acute phase of the disease, but are much less effective in the chronic phase.[3] Furthermore, these drugs can cause serious side effects that limit their use. Due to its toxicity, nifurtimox is no longer used in several countries, such as Brazil.[4] Therefore, the search for safer and more effective trypanocidal drugs is mandatory.

Cruzain, the major cysteine protease of *T. cruzi*, provides an attractive target for rational drug design due to its essential functions for the parasite.[5–8] It shows high residue identity to cysteine proteases of other trypanosomatids,[9] which can be an advantage since there is a possibility of developing a broad-spectrum inhibitor clinically useful against more than one disease.[10,11] Indeed, cruzain shares 70% identity with rhodesain, the major cysteine protease and a relevant target of *Trypanosoma brucei*, which causes African trypanosomiasis or sleeping sickness.[12]

Several classes of cruzain inhibitors have been identified and in some cases shown to be promising trypanocidal agents.[13–17] Through high-throughput screening of a compound library of over 190,000 small molecules, Ferreira et al. identified 146 non-covalent, competitive inhibitors of cruzain. Indole-pyrimidine **1**

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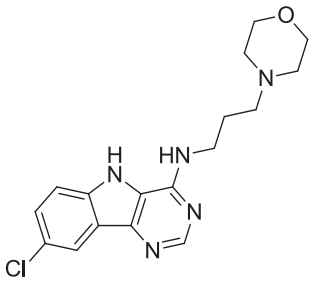

1

Fig. 1. Chemical structure of the indole-pyrimidine **1**.

(Fig. 1) is among one of the most promising compounds found in this screen (K_i = 2.0 μM; IC_{50} = 2.5 μM).[18]

Based on the structure of indole-pyrimidine **1**, we decided to synthesize a novel series of analogues using the molecular simplification strategy to identify groups essential for the activity and to establish structure-activity relationships (SAR).

Considering the three fused rings of **1**, analogues were proposed to determine which rings could be removed or replaced without affecting activity, according to the scheme shown in Fig. 2. Variations of the side chain length, basicity and substituents were also explored (represented by R_1 in Fig. 2). Evaluation of the synthesized compounds in assays with the parasitic and mammalian enzymes and also in assays with *T. cruzi* revealed a novel chemical series which includes selective cruzain and rhodesain inhibitors with trypanocidal activity.

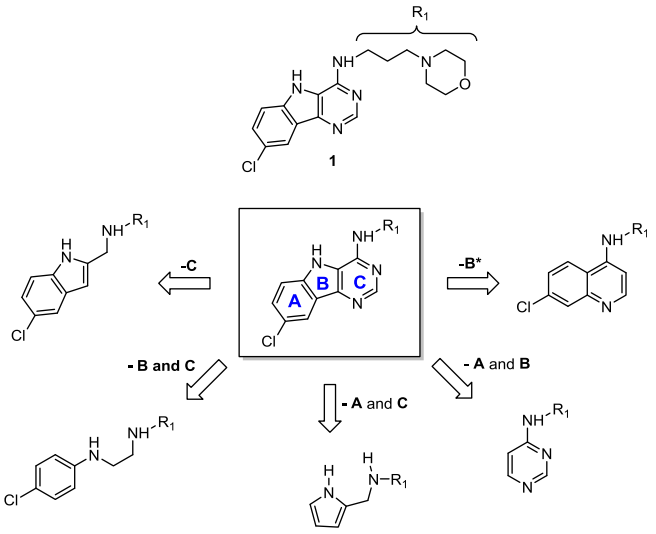

Fig. 2. Simplification strategy employed in the design of analogues of **1**. *Removing ring B and replacing ring C.

2. Results and discussion

2.1. Chemistry

Based on the strategy of molecular simplification, five series of analogues of indole-pyrimidine **1** were obtained: indole (removing ring C), pyrimidine (removing rings A and B), quinoline (removing ring B and replacing ring C), aniline (removing rings B and C), and pyrrole (removing rings A and C) derivatives, as illustrated in Fig. 3. The analogues were obtained in 1–4 steps, with medium to high yields, from easily available starting materials (for details, vide Supplementary Material). Among 50 compounds synthesized, 16 have been described for the first time: **2a**, **2b**, **2c**, **3a**, **3c**, **4a**, **4b**, **4c**, **5k**, **6b**, **6c**, **8a**, **8b**, **8c**, **9b** and **9d**.

2.2. Enzymatic assays

Based on the high sequence identity between cruzain and rhodesain, enzymatic assays were performed with both enzymes, to investigate the possibility of finding a dual inhibitor. All synthesized compounds were evaluated based on kinetic assays in which the fluorescence generated by the cleavage of substrate Z-FR-AMC is monitored. Initial screening was performed with 100 μM of each compound, and IC_{50} values were determined for those that inhibited the enzymes by at least 60% and were soluble at higher concentrations.

Our first attempt to simplify our lead **1** was to remove the pyrimidine ring, therefore obtaining a series of 2-substituted indoles. Substituents containing aliphatic, aromatic or heterocyclic rings were exploited at this position, always maintaining the amino group and the indole separated by one carbon, as observed in the original lead. Corresponding analogues containing a 5-chloroindole were also synthesized. However, all indole derivatives were inactive against both enzymes, even the most similar to the original prototype **3c**, which differed only by removal of the pyrimidine ring (Table 1). These results indicate either the importance of protein-ligand interactions directly involving this ring or its importance to rigidify the molecule and to orient the 3-(morpholin-4-yl) propyl-amino substituent.

Analogues containing a pyrimidine ring linked to three different substituents (**4a-c**) indicate that even though this ring may be important for enzyme inhibition, removal of the indole also leads to inactive compounds. This conclusion is supported by **4c**, which differs from **1** only by an indole removal.

Considering that removal of either indole or pyrimidine rings was not tolerated, next we exploited another simplification strategy based on 4-substituted chloroquinoline derivatives.

First, we synthesized compound **5a**, which contained the same substituent as **1**, our lead. This compound inhibited cruzain (IC_{50} = 15 ± 2 μM), being slightly less potent but considerable more synthetically accessible than **1**. This result motivated the synthesis of quinoline analogues containing substituents which varied in their length, charge and presence of a ring system (phenyl, morpholine or piperidine). Overall, this series was active, as all compounds at 100 μM inhibited by 40–90% the enzymes activities (Table 2). Among those for which IC_{50} could be determined (6 compounds for cruzain and 7 for rhodesain), there was at most a 4-fold difference (compare **5a** vs **5e** against cruzain and **5j** vs **5g** against rhodesain). This data indicates that the SAR exploited was flat within the molecules evaluated, without activity cliffs. However, some SAR trends were observed, which are discussed below.

Compound **5a** was the most potent against cruzain (IC_{50} = 15 ± 2 μM), but **5f** shows very similar potency (IC_{50} = 28 ± 10 μM), while containing a smaller substituent. These compounds share a 3-carbon distance between the aliphatic nitrogen and a heteroatom, and analogues in which this distance was reduced to 2-carbons were 2–3-fold less potent (compare **5f** vs **5e** and **5a** vs **5b**). On the other hand, this trend was not observed against rhodesain.

The most significant difference between inhibition profiles for the two enzymes was observed for compound **5a**, which was the most potent against cruzain while being among the least potent against rhodesain. On the other hand, the most potent rhodesain inhibitor was **5j** (IC_{50} = 34.2 ± 0.1 μM), the derivative containing a