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Design, synthesis, molecular docking and biological evaluation of thiophen-2-iminothiazolidine derivatives for use against *Trypanosoma cruzi*

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ABSTRACT

In this study, we designed and synthesized a series of thiophen-2-iminothiazolidine derivatives from thiophen-2-thioureic with good anti-*Trypanosoma cruzi* activity. Several of the final compounds displayed remarkable trypanocidal activity. The ability of the new compounds to inhibit the activity of the enzyme cruzain, the major cysteine protease of *T. cruzi*, was also explored. The compounds **3b**, **4b**, **8b** and **8c** were the most active derivatives against amastigote form, with significant IC_{50} values between 9.7 and 6.03 μM. The **8c** derivative showed the highest potency against cruzain (IC_{50} = 2.4 μM). Molecular docking study showed that this compound can interact with subsites S1 and S2 simultaneously, and the negative values for the theoretical energy binding (E_b = −7.39 kcal·mol^{-1}) indicates interaction (via dipole–dipole) between the hybridized sulfur sp^3 atom at the thiazolidine ring and Gly66. Finally, the results suggest that the thiophen-2-iminothiazolidines synthesized are important lead compounds for the continuing battle against Chagas disease.

1. Introduction

Neglected tropical diseases (NTDs) are a diverse group of diseases that prevail in tropical and subtropical countries and are responsible for causing illness in more than 1 billion people around the world. It has been estimated that around 8 million of these cases are associated with the parasite *Trypanosoma cruzi*, which causes Chagas disease.[1–3] This disease is controlled at present through the elimination of the vectors with the use of insecticides and the serological screening of blood. Better housing and educational campaigns are also fruitful approaches. As with other parasitic diseases, this pathology is associated with poverty and low educational levels.[4]

Current chemotherapy for Chagas disease is unsatisfactory due to its limited efficacy, particularly in the chronic phase, with frequent side effects that can lead to the discontinuation of treatment.[5] The development of resistance by some strains of *T. cruzi* toward gold-standard drugs, such as nifurtimox and benznidazole (Fig. 1), represents a serious public health problem.[2] Also, these drugs present disadvantages that limit their use: they produce active metabolites which to interact with the DNA of the host leading to deleterious effects, such as cancer[6,7], resistance, lack of efficacy in the late-stage of the disease and a lack of pediatric formulations.[8] The survival of T. cruzi in the cells of the host is guaranteed by important enzymes (e.g. cruzain) which play a role in different processes including absorption, penetration, survival, infectivity, immune evasion, nutrition and growth.[6]

Cruzain is a cathepsin-L-like protease (also known as cysteine protease) of the papain family. It is thought to be essential for

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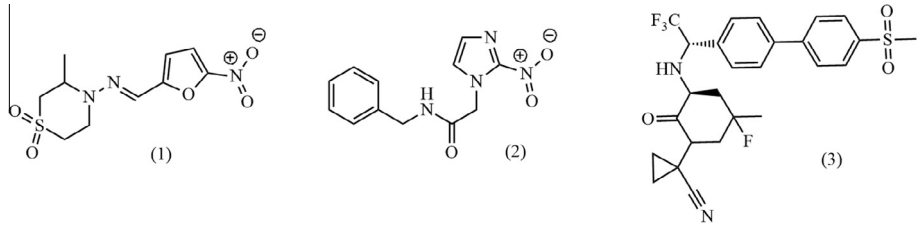

Figure 1. Structures of nifurtimox (1), benznidazole (2) and odanacatib (3).

the infection of host cells and the replication and metabolism of the parasite and it plays multiple roles in the disease pathogenesis.[4,5] Furthermore, the selective inhibition of cysteine proteases as a therapeutic target has transcended the laboratory to the clinic. Recently, odanacatib (Fig. 1), a drug based on cysteine protease inhibition, has shown high efficacy and a good safety profile in Phase III clinical trials. Also, many studies in animal models have validated the use of this enzyme for the control and elimination of *T. cruzi*.[8]

Many thiophene compounds have been described as very active derivatives against *T. cruzi* (Fig. 2) and these are interesting starting materials for new therapeutic agents.[9] The thiophene aromatic ring is a hydrophobic site which binds to the hydrophobic-pockets in some enzymes.[10]

The pharmacological activity of thiazolidine compounds is of current interest. Thiazolidine and its hybrids have been reported to possess promising anticancer and antiviral properties. In addition, the thiazolidine scaffold is extremely important in the design and synthesis of novel biologically active agents with action against *Trypanosoma* spp.[11] Many compounds with the thiazolidine ring in their structure (Fig. 3) have been described in the literature and they show promising anti-*T. cruzi* activity.[1,5,6,11]

In this study, we synthesized new thiophene-thiazolidine hybrid derivatives bearing various groups at the thiazolidine ring, with a linker (imine group) between the thiophene and thiazolidine rings (Fig. 4). All derivatives were tested for their ability to inhibit the in vitro growth of amastigote and trypomastigote forms of *Trypanosoma cruzi* and the activity of the enzyme cruzain. In addition, we carried out theoretical studies involving molecular docking simulations and pharmacophoric identification.

2. Results and discussion

2.1. Chemistry

The 2-aminothiophene analogues (**1**, **2**) were readily prepared via the Gewald reaction, a multicomponent synthesis involving an aldehyde or ketone, an activated nitrile and elemental sulfur.[12–14] The intermediates (**3a–d**) were then synthesized through the treatment of **1** or **2** with substituted isothiocyanates (phenyl and allyl). Spectral analysis for these compounds was carried out according to methods described in the literature.[13,15] Finally, thiophen-2-iminothiazolidines were prepared via thia-Michael cyclization (**7a–d**) or substitution followed by intramolecular cyclization (**4a–d**, **5c**, **d**, **6a**, **6c** and **8a–d**) between thiophen-2-thiourea and dielectrophiles.[16,17] All of these reactions proceeded well with refluxing overnight. The synthetic route is shown in Scheme 1. Four compounds previously designed were not included in this work (two cyclization using ethyl 2-chloro acetate and two using 2-bromomalonate) due the formation of many secondary products and impossibility of purification by recrystallization or flash column chromatography.

The structures of the new compounds synthesized were established by ^{1}H and ^{13}C 1D and 2D NMR analysis. For the final compounds, in ^{1}H NMR spectra, signs of the protons from thiourea moiety of **3a–d** signs were not displayed, and peaks resonated at 154.48–158.49 and 158.32–163.94 ppm in the ^{13}C NMR spectra were respectively assigned to C-4 and C-2 of the thiazolidine ring. For compounds **4a–d** the ^{1}H NMR spectra revealed the presence of a singlet for thiomethylene protons at 3.66–3.90 ppm. In addition, ^{13}C NMR spectra exhibited resonated peaks for the same moiety at 33.39–34.97 ppm, as well as signals at 155.86–158.49 and

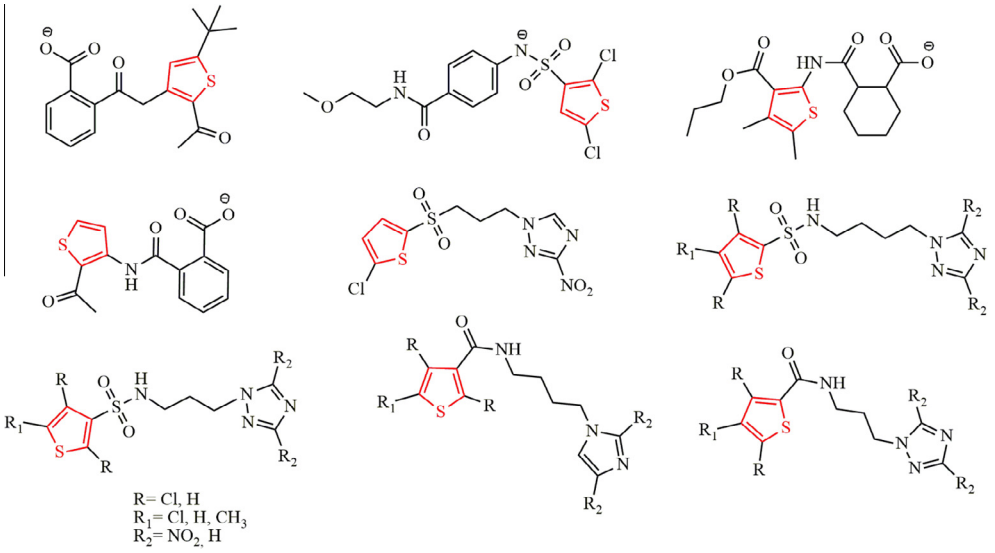

Figure 2. Some thiophene derivatives described in the literature which are active against *Trypanosoma cruzi*.