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Original article

2-Pyridyl thiazoles as novel anti-*Trypanosoma cruzi* agents: Structural design, synthesis and pharmacological evaluation

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ABSTRACT

The present work reports on the synthesis, anti-*Trypanosoma cruzi* activities and docking studies of a novel series of 2-(pyridin-2-yl)-1,3-thiazoles derived from 2-pyridine thiosemicarbazone. The majority of these compounds are potent cruzain inhibitors and showed excellent inhibition on the trypomastigote form of the parasite, and the resulting structure–activity relationships are discussed. Together, these data present a novel series of thiazolyl hydrazones with potential effects against Chagas disease and they could be important leads in continuing development against Chagas disease.

1. Introduction

Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) [1]. About 7–8 million people worldwide are estimated to be infected with *T. cruzi*, mainly in Latin America.

Over the years, numerous chemical classes against *T. cruzi* have become available, but there is still no effective treatment for all phases of the disease [2]. Recent research has pointed to the ergosterol biosynthetic pathway as a biochemical target [3,4]. Azole derivatives such as posaconazole and ravuconazole, have been tested and presented trypanocidal activity, but they are not yet available as therapeutics [5–7].

Despite the efforts of many investigators in the research of a new anti-Chagas drugs, only two drugs are currently used to treat it, nifurtimox and benznidazole [6,8] (Fig. 1). 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 discontinuation of treatment.

Among a number of drug targets being investigated for Chagas disease, cruzain, the major cysteine protease active in the parasite, is a prominent candidate [9–12]. Cruzain is a cathepsin-L-like protease of the papain family thought to be important for intracellular replication and differentiation of the *T. cruzi* parasite [13]. Among the chemical groups explored for anti-Chagas activity, thiazolyl hydrazones are noteworthy because of their wide biological, especially anti-parasitic, activities [14–17].

In 2004, Greenbaum et al. observed that thiosemicarbazones with a pyridyl moiety inhibit cruzain catalytic activity [18]. Subsequent studies have demonstrated the trypanocidal activity of thiosemicarbazones and their metal complexes. Recently, Caputo et al. have demonstrated trypanocidal activity for a series of 4-arylthiazolylhydrazones [19], with a broad and potent activity for all forms of the parasite. Recently studies have reported inhibition

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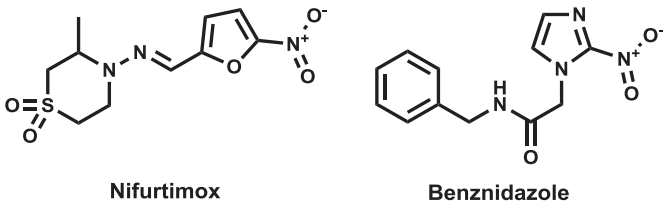


Fig. 1. Structures of nifurtimox and benznidazole.

of the cysteine protease cruzain by thiosemicarbazones [16]. Our efforts toward new antichagasic drug since 2010 have led us to a variety of thiosemicarbazones and thiazolyl hydrazones as trypanocidal agents [16,19–22]. The promising results achieved by compounds bearing a thiazole ring motivated us to investigate the trypanocidal activity of novel thiazolyl hydrazones derived from 2-pyridyl thiosemicarbazone, with changes being made in the phenyl ring attached in N2. In continuation of our search for bioactive molecules, we envisaged that the derivatization of the thiosemicarbazone group into thiazole moiety would generate novel templates, which are likely to exhibit anti-*T. cruzi* activity. We also investigate pyridines as trypanocidal agents because their wide applicability in organic synthesis, low price and facility in synthesis. Here, these compounds were tested *in vitro* against *T. cruzi* parasite epimastigote and trypomastigote forms, and cruzain protease. In this synthetic design of a structure–activity relationship (SAR) library, attention was paid to further explore substituents around the phenyl ring attached in thiazole ring (C9). This study generated basic SARs about tripomastigote form and cruzain enzyme around substituents in phenyl ring, and using the scaffold shown in Scheme 1. Specifically, we report the preparation of twenty-four 2-(pyridin-2-yl)-1,3-thiazoles (**3**–**27**) by ultrasound-assisted synthesis. The synthesized compounds were characterized by IR, NMR and mass spectral studies. The 2-(pyridin-2-yl)-1,3-thiazoles were assayed for their *in vitro* anti-*T. cruzi* activity against the epimastigote and trypomastigote forms of the parasite. Their cytotoxicity in mammalian cell cultures was also investigated. Further investigations on the possible involvement of 2-(pyridin-2-yl)-1,3-thiazoles with cruzain activity as potential therapeutic targets were performed.

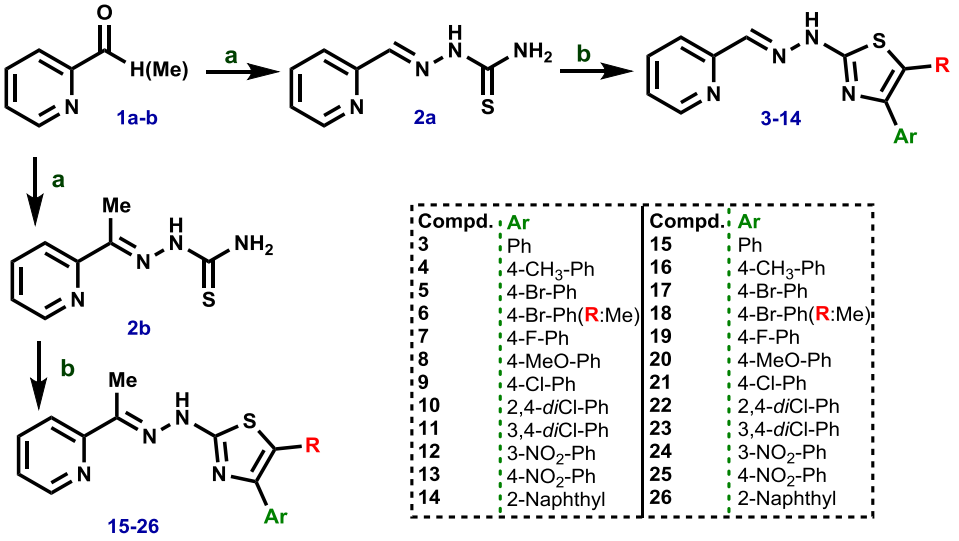
2. Results and discussion

2.1. Chemistry

2-(pyridin-2-yl)thiosemicarbazones (**2a**–**b**) were prepared by reacting commercially available thiosemicarbazides with the appropriate aldehyde or ketone (1:1.2 mol ratio) via Schiff base condensations using ultrasound irradiation in presence of a catalytic amount of AcOH. This reaction condition led to high yields (80–90%). 2-(pyridin-2-yl)-1,3-thiazoles (**3**–**26**) were prepared via Hantsch cyclization between 2-(pyridin-2-yl)thiosemicarbazones (**2a**–**b**) and substituted 2-bromoacetophenones (Scheme 1). These reactions proceed well upon refluxing with ethanol (2–4 h), but here we adapted this to ultrasound conditions at room temperature [23] using 2-propanol as solvent [24]. This resulted in good yields (50–85%) and shorter reaction times (60 min in most cases) compared with the reflux protocol.

Microanalysis and NMR data are compatible with the proposed compounds. In theory, two geometrical isomers (*E* and *Z*) about the imine (C=N) double bond are possible for the thiosemicarbazones. However, analysis of the ^{1}H NMR spectra of the target compound indicated one predominant isomer; the *E* isomer by comparison with known analogues [25]. Intramolecular H-bonding involving the proton attached to N4 (in DMSO) with the imine N-atom leads to a distinctive singlet around 10.2 ppm [25] and this is also seen here.

Once thiosemicarbazones were characterized, the respective 2-(pyridin-2-yl)-1,3-thiazoles were characterized by usual spectroscopy. As exemplified with the ^{1}H NMR analysis of (2-(1-(pyridin-2-yl)ethylene)hydrazinyl)-4-phenyl-1,3-thiazole (**15**), the singlet peak at δ 2.43 corresponds to the methyl group. The aromatic protons occurred as doublets or triplets. For the pyridyl ring, peaks were observed at δ 7.76, 8.23, 8.35 and 8.73. For the aromatic ring coupled to the thiazole ring, doublet and triplet peaks were found at δ 7.30, 7.39 and 7.85. For the thiazole ring, a singlet at δ 7.44 was found. In addition, the NH proton appeared as broad singlet at δ 5.53. The ^{13}C NMR spectrum of (**15**) the ^{13}C=S resonance from the parent thiosemicarbazone disappeared while a new ^{13}C–H resonance at ~106 ppm appeared, confirming cyclization in addition to the resonance at ~169 ppm. Quaternary carbon peaks were confirmed by DEPT experiments to appear at δ 134.2, 140.1, 150.0 and 168.9. Peaks of the pyridine aromatic carbons were found at



Compd.	Ar	Compd.	Ar
3	Ph	15	Ph
4	4-CH_3-Ph	16	4-CH_3-Ph
5	4-Br-Ph	17	4-Br-Ph
6	4-Br-Ph(R:Me)	18	4-Br-Ph(R:Me)
7	4-F-Ph	19	4-F-Ph
8	4-MeO-Ph	20	4-MeO-Ph
9	4-Cl-Ph	21	4-Cl-Ph
10	2,4-*di*Cl-Ph	22	2,4-*di*Cl-Ph
11	3,4-*di*Cl-Ph	23	3,4-*di*Cl-Ph
12	3-NO_2-Ph	24	3-NO_2-Ph
13	4-NO_2-Ph	25	4-NO_2-Ph
14	2-Naphthyl	26	2-Naphthyl

Scheme 1. Synthetic procedures for thiosemicarbazones (**2a**–**b**) and 2-(pyridin-2-yl)-1,3-thiazoles (**3**–**26**). Reagents and conditions: (a) thiosemicarbazide, 2-propanol, acetic acid (3 drops), ultrasound irradiation, r.t., 120 min; (b) substituted 2-bromoacetophenones, 2-propanol, $CaCO_3$, ultrasound irradiation, r.t., 60 min; *R: H for all compounds, except to (**6**) and (**18**) where R: Me.