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Synthesis of pyrazoline–thiazolidinone hybrids with trypanocidal activity

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ABSTRACT

A series of novel 4-thiazolidinone–pyrazoline conjugates have been synthesized and tested for anti-*Trypanosoma brucei* activity. Screening data allowed us to identify five thiazolidinone–pyrazoline hybrids, which possess promising trypanocidal activity, with $IC_{50} \leq 1.2$ μM. The highest active thiazolidinone–pyrazoline conjugates **3c** and **6b** (IC_{50} values of 0.6 μM and 0.7 μM, respectively) were 6-times more potent antitrypanosomal agents than nifurtimox. In addition, these compounds, as well as **6d** and **6e** had selectivity index higher than 50, and were more selective than nifurtimox. SAR study included substituent variations at the pyrazoline moiety, modifications of *N3* position of the thiazolidinone portion, elongation of the linker between the heterocycles, as well as rhodanine–isorhodanine isomerism. It was also shown that methyl or aryl substitution at the thiazolidinone *N3*-position is crucial for trypanocidal activity.

1. Introduction

Human African trypanosomiasis (HAT) is among the most serious neglected tropical diseases and is caused by *Trypanosoma brucei gambiense* and *rhodesiense* (West and East African forms, respectively). It is estimated that about 20 000 people are currently suffering from HAT [1]. Available treatment still mainly relies on the drugs developed many decades ago, which in their majority are toxic, show decreasing efficacy, and involve administration/patient compliance issues. Therefore, the discovery and development of novel effective, safe, and affordable antitrypanosomal agents is a task of high priority.

The pharmacological activities of thiazolidinone [2–4] and pyrazoline [5,6] derivatives are of current interest. Thiazolidinone–diazole hybrids have been reported to possess promising chemotherapeutic properties including anticancer [7–14] and antiviral [15–17] activities. On the other hand, thiazolidinones and pyrazolines (Fig. 1) are of great importance in the design and synthesis of novel biologically active agents that exert trypanocidal activity [18–26]. Leite et al. [18] tested a small library of aryl-4-oxothiazolylhydrazones against *Trypanosoma cruzi*-infected cells. Docking studies suggested that these compounds were potential ligands for the cysteine protease cruzain. The most promising compound **I**, has shown to be very active (IC_{50} (Y strain) = 0.3 μM) at non-cytotoxic concentrations towards mammalian cells, and its potency was comparable to the reference drugs nifurtimox and benznidazole [18]. The 2-hydrazolyl-4-thiazolidinone-5-carboxylic acid derivatives **II** have shown promising activity on the cruzipain protease [19].

The 4-thiazolidinone derivatives with 4-dialkylaminobicyclo [2.2.2]octane fragment **III** were tested against *T. brucei rhodesiense* and showed moderate to weak activity ($IC_{50} > 6.12$ μM) [20]. The 2-thioxo-4-thiazolidinone-3-acetic acid derivatives **IV** were identified as inhibitors of *T. brucei* dolicholphosphate mannose synthase and the most active compounds demonstrated trypanocidal activity against cultured bloodstream-form *T. brucei* (ED_{50} from 96 to 492 μM) [21]. It should be noted that fused and non-fused thiazolidinone derivatives were also tested for their trypanocidal activity. Thiopyrano[2,3-*d*][1,3]thiazoles, which can be considered as cyclic isosteric mimetics of their synthetic precursors 5-arylidene-4-thiazolidinones without Michael accepting functionalities, have been evaluated as potential antitrypanosomal agents [22]. The most active analogue **V** inhibited *T. brucei brucei* and *T. brucei gambiense* with an IC_{50} of 0.26 and 0.42 μM,

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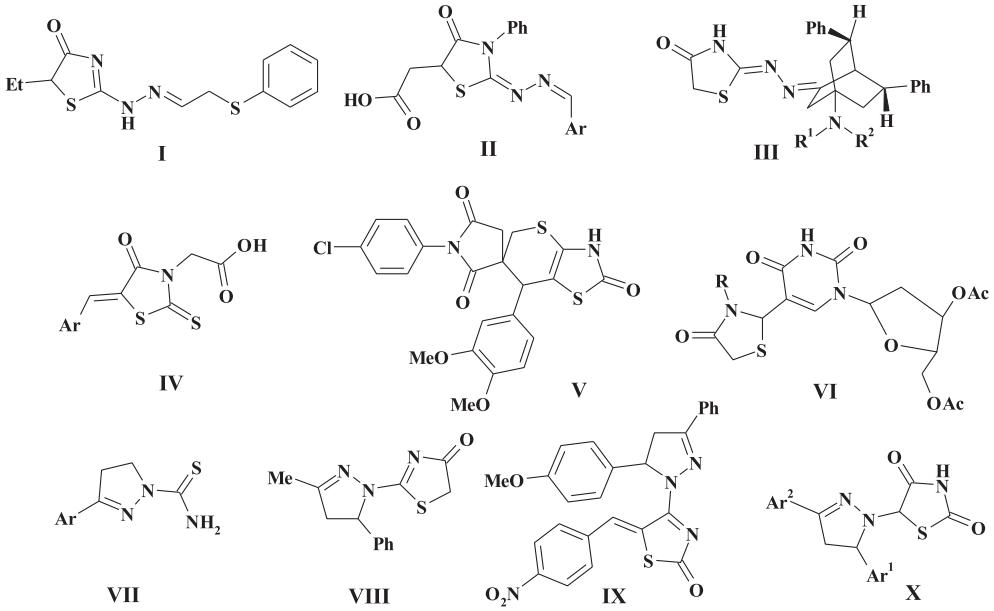

Fig. 1. Thiazolidinones and pyrazolines with trypanocidal activity.

respectively. It has been also reported that pyrimidine nucleoside-thiazolinin-4-one hybrids **VI** possess moderate activity against bloodstream-form *T. brucei brucei* with IC_{50} of 25–>100 μM [23].

In the case of the pyrazoline derivatives, some novel compounds (e.g. **VII)** have been identified as inhibitors of the trypanosomal cysteine protease cruzain with IC_{50} of 40–230 nM [24]. Therefore the combination of thiazolidinone and pyrazoline fragments in one molecule is a perspective approach to design promising anti-trypanosomal agents (Fig. 1). Thus, among the thiazolidinone–pyrazoline conjugates, compound **VIII** was investigated for its activity against some causative organisms of tropical diseases and showed the highest activity against *T. brucei rhodesiense* (IC_{50} = 12 μg/ml) [25].

From our previous studies on the *in vitro* antitrypanosomal activity of non-condensed thiazolidine–pyrazoline derivatives, compound **IX** has identified as a hit agent against *T. brucei brucei* (IC_{50} = 3.01 μg/ml) [26]. To continue this study the 5-pyrazoline substituted 4-thiazolidinones **X** were further selected in *in vitro* assays against *T. brucei brucei* and *T. brucei gambiense* and showed IC_{50} from 5.4 to 13.9 μM and 2.5 to 6.7 μM, respectively [16].

In the present study, we designed (Fig. 2) and synthesized new thiazolidinone–pyrazoline hybrid compounds bearing various substituents at the thiazolidinone *N3* position and pyrazoline ring 3 and 5 positions, and having different linkages between the pyrazoline ring and the thiazolidinone scaffold. The resulting derivatives were tested for their ability to inhibit the *in vitro* growth of *T. brucei gambiense*. The chemical modifications of 5-pyrazoline substituted 4-thiazolidinones resulted in a 4–10-fold increase in potency (for the most active candidates) as compared to the pyrazoline–thiazolidinone analogues **X** [16].

2. Results and discussion

2.1. Chemistry

The general methods for the synthesis of the new thiazolidinone–pyrazoline hybrids are depicted in Schemes 1 and 2.

The synthesis of 5-ethoxymethylidene rhodanine **1a** was effected by reaction of 2-thioxo-4-thiazolidinone (rhodanine) with triethyl orthoformate [27]. Considering the critical influence of the thiazolidinone *N3*-substituent for the trypanocidal activity [28], the corresponding 5-ethoxymethylene *N3*-substituted rhodanines with methyl (**1b**), furan-2-ylmethyl (**1c**) and aryl (**1d** and **1e**) groups were synthesized in the same conditions. The target thiazolidinone–pyrazoline hybrids **2a**–**e**, **3a**–**c**, **4a**–**d**, **5a**–**e**, and **6a**–**g** with a methylidene linking group were obtained by reacting 5-ethoxymethylene rhodanines **1a**–**e** with the appropriate 3,5-diaryl-2-pyrazolines in refluxing ethanol (Scheme 1). In order to investigate whether the migration of the thiocarbonyl function from *C2* to *C4* position of the thiazolidine ring is crucial for activity, the 4-thioxothiazolidin-2-one-pyrazoline conjugates **7a**–**b** were synthesized by using 4-thioxo-2-thiazolidinone (isorhodanine) as starting material (Scheme 1).

To study the influence of the linking group on the trypanocidal activity of the thiazolidinone–pyrazoline conjugates, the carboxylic acid derivative **8** was synthesized by reaction of **1b** with glycine in acetic acid. The coupling of **8** with 3,5-diaryl-2-pyrazolines in the presence of DCC led to the 2-oxoethylaminomethylene-linked thiazolidinone–pyrazoline hybrids **9a**–**c** (Scheme 2).

The structure of the newly synthesized heterocyclic-substituted thiazolidinones was confirmed by elemental analysis and spectroscopic data (^{1}H NMR, ^{13}C NMR and LCMS). ^{1}H NMR spectra of all synthesized compounds showed characteristic patterns of an AMX system for the protons at positions 4 and 5 of the pyrazoline ring. The olefinic proton (=CH) of compounds **2a**–**e**, **3a**–**c**, **4a**–**d**, **5a**–**e** and **6a**–**g** showed a singlet at δ ~ 7.19–7.95 ppm. In the ^{1}H NMR spectra of **2a**–**c**, **7a** and **7b**, the broad singlet of the NH proton of the thiazolidinone ring appeared at δ ~12.32–12.97 ppm.

Due to the enamine fragment at 5-position of the thiazolidinone ring, compounds **8** and **9a**–**c** exist as a mixture of *Z* and *E* isomers (Fig. 3). In the ^{1}H NMR spectra of compound **8**, the olefinic proton (=CH) resonates as two doublets at δ = 7.63 ppm (*Z*-isomer) and δ = 7.43 ppm (*E*-isomer). In addition, the enamine NH proton appeared as two multiplets at δ = 8.93–8.97 ppm (*E*-isomer) and δ = 8.28–8.31 ppm (*Z*-isomer). Two similar multiplets for the enamine NH proton were also observed in the ^{1}H NMR spectra of compounds **9a**–**c**.