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Synthesis and antitrypanosomal activity of new 6,6,7-trisubstituted thiopyrano[2,3-*d*][1,3]thiazoles

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ABSTRACT

A series of novel 6,6,7-trisubstituted thiopyrano[2,3-*d*][1,3]thiazoles-based molecules have been synthesized and evaluated as potential antitrypanosomal agents. The most active analogue **3b** inhibited *Trypanosoma brucei brucei* and *Trypanosoma brucei gambiense* with an IC_{50} of 0.26 and 0.42 μM, respectively. They could be considered as potent hits for further antitrypanosomal drug discovery efforts.

Human African trypanosomiasis (HAT, African sleeping sickness) is a fatal infectious disease caused by the protozoa parasite *Trypanosoma brucei* and transmitted by the tsetse fly. Sixty millions people are at risk of this infection, which in West Africa is caused by *Trypanosoma brucei gambiense* while in East Africa by *Trypanosoma brucei rhodesiense*. Cattle act a reservoir for HAT and can be infected by the subspecies *Trypanosoma brucei brucei*, *Trypanosoma congolense* and *Trypanosoma evansi*, which have high economical impacts on local populations. Drugs therapy is a cornerstone of trypanosomicidal treatment. For the early stages of the disease suramin and the diamidine pentamidine are commonly used. Arsenical melarsoprol and eflornithine are reserved for the late stages of disease when the central nervous system is affected. Except for eflornithine, which has better safety profile, the other drugs cause many side effects and can induce severe toxicity reaction. Importantly, eflornithine is not effective in *T. b. rhodesiense* associated disease. In addition to toxicity issues, the problems of drug resistance have been reported for pentamidine, melarsoprol and eflornithine.[1] Thus, there is an urgent need for the development of the new drugs against this devastating illness.

It is well known that 4-thiazolidinones and related heterocyclic systems are important scaffolds in drug discovery. Their derivatives were demonstrated with antiinflammatory, antitumor, antimicrobial, antidiabetic and antibacterial actions.[2] Recently, thiazolidinones derivatives have been identified as perspective agents in antitrypanosomal treatment, in particular among 2-hydrazono-4-thiazolidinones.[3,4] Moreover 5-benzylidene-2-thioxo-4-thiazolidinone-3-acetic acids were reported as first small molecule inhibitors of *T. brucei* dolicholphosphate mannose synthase (DPMS), a validated drug target in African sleeping sickness.[5] However, 5-arylidene-4-thiazolidinones can be considered as electrophilic and potentially reactive substances due to possible Michael addition of the nucleophilic protein residues to the exocyclic double bond. This property characterizes 5-arylidene-4-thiazolidinones as the frequent hitters or pan assay interference compounds (PAINS) that are useless in the modern drug discovery process because of their low selectivity.[6] Our previous research demonstrated a number of arguments in favor of 7-arylthiopyrano[2,3-*d*][1,3]thiazoles design which could be one of approaches to address the selectivity issue.[7,8] Thiopyrano[2,3-*d*][1,3]thiazoles can be considered as cyclic isosteric mimetics of their synthetic precursors 5-arylidene-4-thiazolidinones without Michael accepting functionalities (Fig. 1). The aim of present letter was to explore the antiparasitic activity of new thiopyrano[2,3-*d*][1,3]thiazoles against *Trypanosoma brucei brucei* (*Tbb*) and *Trypanosoma brucei gambiense* (*Tbg*).

The general synthetic pathways yielding target thiopyrano[2,3-*d*][1,3]thiazoles are shown in Schemes 1 and 2. Itaconic acid and its imides were studied as dienophiles in *hetero*-Diels–Alder reaction with 5-arylidene-4-thioxo-2-thiazolidinones **1a–g** (5-arylideneisorhodanines)[9] as heterodienes. Diene synthesis reactions were performed in glacial acetic acid medium with catalytic amount of hydroquinone to prevent polymerization processes.[10,11] As a

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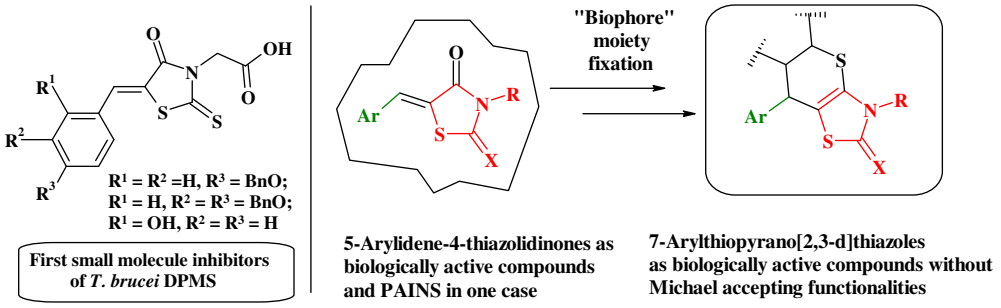
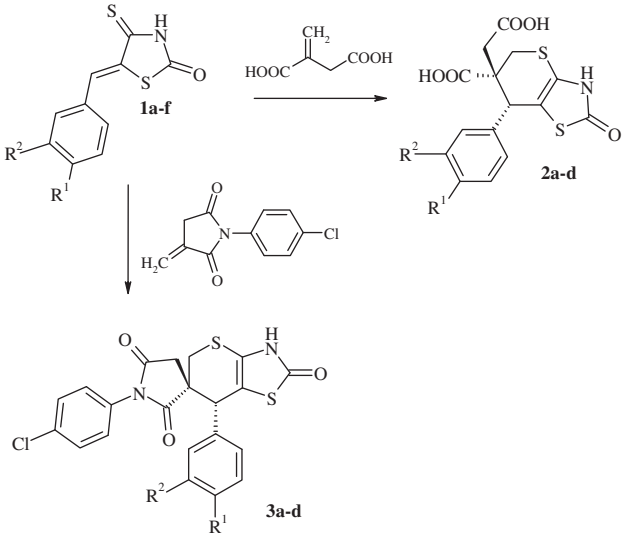
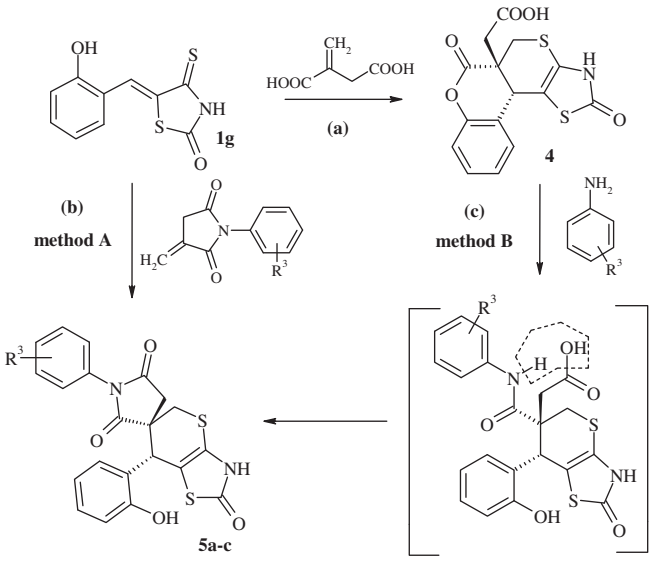


Figure 1. Background for target compounds synthesis.



Scheme 1. Synthesis of *rel*-(6*R*,7*R*)-7-aryl-6-carboxymethylene-2-oxo-3,5,6,7-tetrahydro-2*H*-thiopyrano[2,3-*d*][1,3]thiazole-6-carboxylic acids (**2a-d**) and *rel*-(6′*R*,7′*R*)-3′,7′-dihydro-*2H*,*2*′*H*,*5H*-spiro[pyrolidin-3,6′-thiopyrano[2,3-*d*]thiazol]-2,2′,5-triones (**3a-d**). Reagents and conditions: **1** (1.0 equiv), appropriate dienophile (1.2 equiv), hydroquinone, AcOH, reflux, 2 h, 44–73%.



Scheme 2. Synthesis of *rel*-2-[(5a*R*,11b*R*)-2,6-dioxo-3,5a,6,11b-tetrahydro-*2H*,*5H*-cromeno[4′,3′:4,5]thiopyrano[2,3-*d*]thiazol-5a-yl]acetic acid (**4**) and *rel*-(6′*R*,7′*R*)-7′-(2-hydroxyphenyl)-1-aryl-3′,7′-dihydro-*2H*,*2*′*H*,*5H*-spiro[pyrolidin-3,6′-thiopyrano[2,3-*d*]thiazol]-2,2′,5-triones (**5a-c**). Reagents and conditions: (a) **1g** (1.0 equiv), itaconic acid (1.2 equiv), hydroquinone, AcOH, reflux, 2 h, 45%; (b) **1g** (1.0 equiv), appropriate itaconimide (1.2 equiv), hydroquinone, AcOH, reflux, 2 h, 42–45%; (c) **4** (1 equiv), appropriate amine (1.3 equiv), AcOH, reflux, 10 h, 32–40%.

result the novel *rel*-(6*R*,7*R*)-7-aryl-6-carboxymethyl-2-oxo-3,5,6,7-tetrahydro-2*H*-thiopyrano[2,3-*d*][1,3]thiazole-6-carboxylic acids (**2a–d**) and *rel*-(6′*R*,7′*R*)-3′,7′-dihydro-*2H*,*2*′*H*,*5H*-spiro[pyrolidin-3,6′-thiopyrano[2,3-*d*]thiazol]-2,2′,5-triones (**3a-g**, **5a–c**) were synthesized. Variant of tandem acylation-*hetero*-Diels–Alder reaction was observed employing 5-(2-hydroxyphenylmethylidene)isorhodanine **1g** as heterodiene 'building block' with itaconic acid, yielding *rel*-2-[(5a*R*,11b*R*)-2,6-dioxo-3,5a,6,11b-tetrahydro-*2H*,*5H*-cromeno[4′,3′:4,5]thiopyrano[2,3-*d*]thiazol-5a-yl]acetic acid **4.**[11] Based on this heterocyclic acid we proposed a counter synthesis method of spiroderivatives **5a–c**.[12,13] Thus, **4** undergo recyclization in the presence of aromatic amines at 100 °C in acetic acid yielding abovementioned thiopyrano[2,3-*d*]thiazole derivatives **5a-c**. Study of ESI-MS, homonuclear ^{1}H and ^{13}C NMR spectra, COSY and NOESY (1D with CH-Ar proton) experiments as well as heteronuclear HSQC and HMBC experiments confirmed the structures of synthesized compounds and the relative stereochemistry of [2 + 4]-cycloaddition and the consecutively stereoconfiguration of substitutions at the positions 6 and 7, in particular trans positioning of 6-carboxymethylene substituent relatively to 7-aryl moiety. For compound **2c** NOE coupling of CH proton at 3.71 ppm with the protons at 6.94 ppm (12%), 2.94 ppm (8%) and 2.84 ppm (4%) is in the accordance with shown structure, which is present as a racemic mixture. Moreover, the conclusion about stereochemistry of the mentioned itaconic acid based *hetero*-Diels–Alder reactions is consistent with our previous data of X-ray analysis of related 2-[7-(3,5-dibromo-2-hydroxyphenyl)-6-ethoxycarbonyl-2-oxo-5*H*-2,3,6,7-tetrahydro-thiopyrano[2,3-*d*][1,3]thiazol-6-yl]acetic acid.[14]

Compounds **2a–d**, **3a–d** and **5a–c** were studied for their antitrypanosomal activity against *T. brucei* and the results are summarized in Tables 1 and 2. Bloodstream forms of *Tbb* strain 90-13 and *Tbg* strain Feo were cultured in HMI9 medium supplemented with 10% FCS at 37 °C under an atmosphere of 5% CO_2.[15] In all experiments, log-phase cell cultures were harvested by centrifugation at 3000×*g* and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells as described previously.[16] Compounds were first tested at concentrations of 10 and 1 μg/mL (Table 1) and IC_{50}s were determined[17] for the most active ones (Table 2).

Some synthesized thiopyrano[2,3-*d*]thiazole spiroimides exhibited significant inhibitory activity against *T. brucei* as exemplified by **3b**, **3c** and **3d**. Among them the *rel*-(6′*R*,7′*R*)-7′-(3,4-dimethoxyphenyl)-1-(4-chlorophenyl)-3′,7′-dihydro-*2H*,*2*′*H*,*5H*-spiro[pyrolidin-3,6′-thiopyrano[2,3-*d*]thiazol]-2,2′,5-trione **3b** exhibited the most potent activity with an IC_{50} of 0.26 and 0.42 μM against *Tbb* and *Tbg*, respectively. In general, these compounds were slightly more active on *Tbg* than on *Tbb*.

The maleimide fragment in spiroimide active hits (**3b–d**) is perpendicularly distorted due to spiro-attachement to the main