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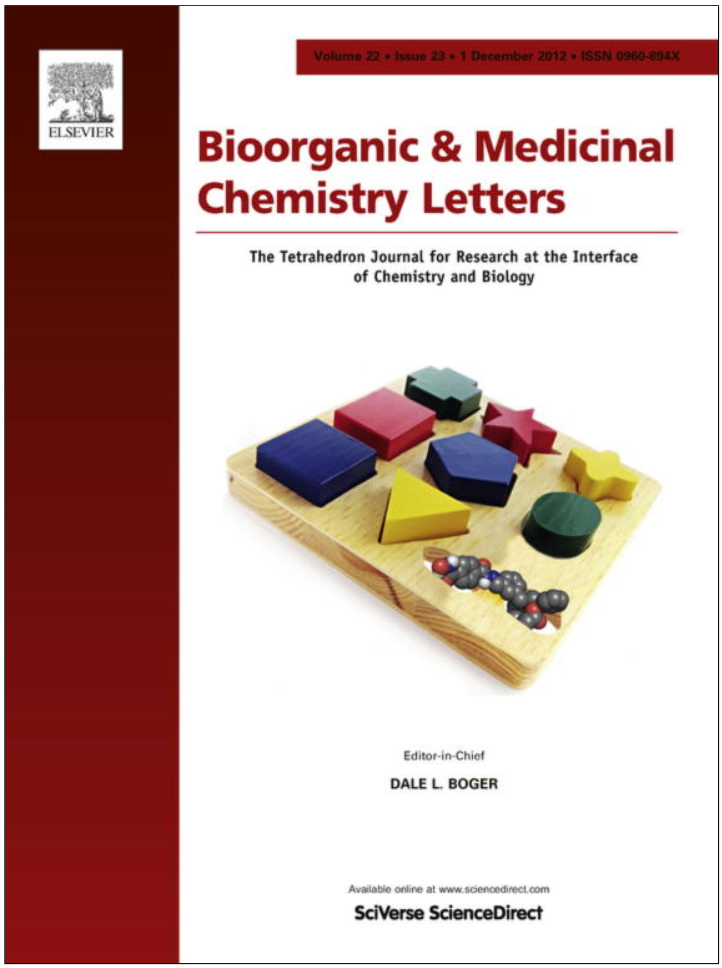
ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · OCTOBER 2012

Impact Factor: 2.42 · DOI: 10.1016/j.bmcl.2012.09.091 · Source: PubMed



Bioorganic & Medicinal Chemistry Letters 22 (2012) 7071–7074

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

Nataliya Zelisko [a], Dmytro Atamanyuk [a,1], Olexandr Vasylenko [b], Philippe Grellier [c], Roman Lesyk [a,*]

[a] Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, 69 Pekarska, Lviv 79010, Ukraine
[b] Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Science of Ukraine, 1 Murmanska, Kyiv 02094, Ukraine
[c] National Museum of Natural History, UMR 7245 CNRS-MNHN, Team APE, CP 52, 57 Rue Cuvier, Paris 75005, France

ARTICLE INFO

Article history:
Received 11 July 2012
Revised 21 September 2012
Accepted 25 September 2012
Available online 2 October 2012

Keywords:
Human African trypanosomiasis
Thiopyrano[2,3-*d*][1,3]thiazoles
Trypanosoma brucei
Antitrypanosomal activity

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

* Corresponding author. Tel.: +38 0322 75 59 66; fax: +38 0322 75 77 34.
E-mail address: dr_r_lesyk@org.lviv.net (R. Lesyk).
[1] Present address: Mutabilis, 102 Avenue Gaston Roussel, Romainville 93230, France.

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http://dx.doi.org/10.1016/j.bmcl.2012.09.091

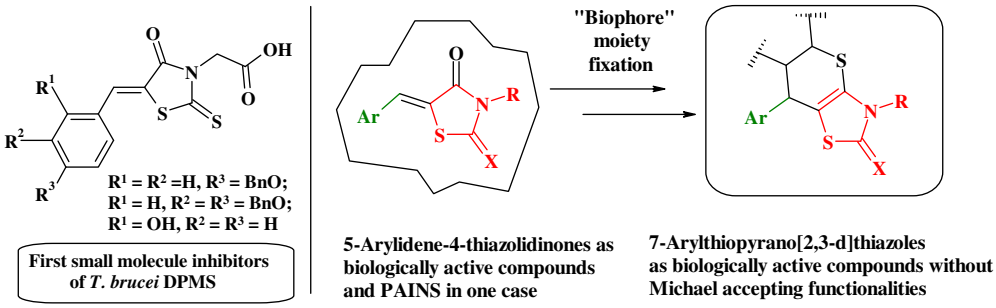
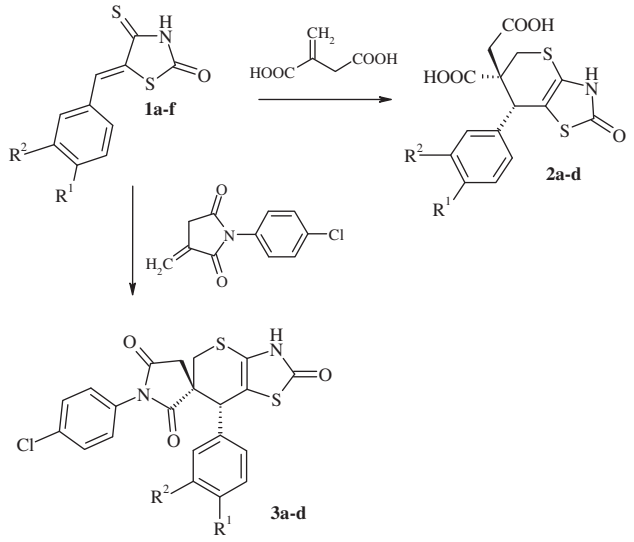
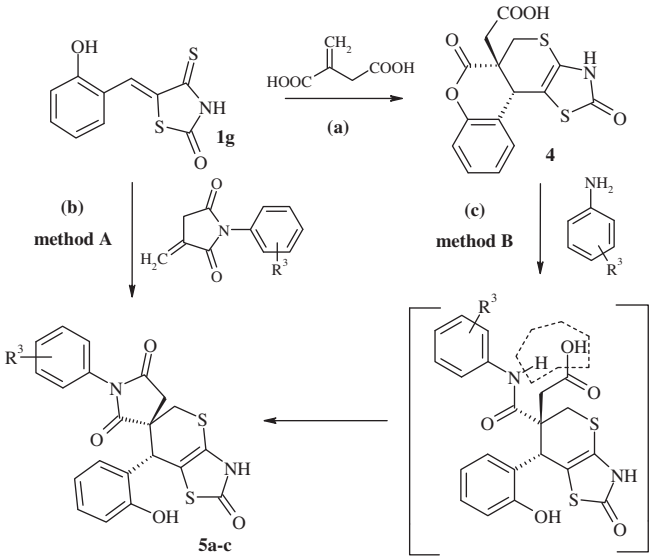


Figure 1. Background for target compounds synthesis.



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-tetrahydrothiopyrano[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

Table 1
Thiopyrano[2,3-*d*]thiazole derivatives (**2a–d**, **3a–d**, **5a–c**), % inhibition of *Tbb* and *Tbg* bloodstream forms at 10 and 1 μg/mL concentrations. nd: not determined

Compound	R^1	R^2	R^3	*Tbb* % inhibition		*Tbg* % inhibition	
				10 μg/mL	1 μg/mL	10 μg/mL	1 μg/mL
2a	BnO	MeO	—	<5	18,1	nd	nd
2b	F_2CHO	MeO	—	<5	11,3	nd	nd
2c	Me_2N	H	—	<5	5,3	nd	nd
2d	Et_2N	H	—	5,6	<5	nd	nd
3a	MeO	H	—	13,0	10,6	9,8	2,3
3b	MeO	MeO	—	93,6	82,4	nd	nd
3c	Et_2N	H	—	98,2	21,1	nd	nd
3d	Me_2N	H	—	98,2	36,8	nd	nd
5a	—	—	4-Cl	11,5	13,4	19,3	0,9
5b	—	—	4-OH	18,5	7,0	15,4	<5
5c	—	—	3-Me	24,5	5,5	15,3	<5

Table 2
IC_{50} of hit-compounds **3b–d** against *Tbb* and *Tbg*

Compound	*Tbb*		*Tbg*	
	IC_{50} (μM)	SD	IC_{50} (μM)	SD
3b	0,2624	0,0139	0,4234	0,1590
3c	2,3985	0,4123	3,6181	0,9259
3d	1,2751	0,3230	1,4711	0,0884
Pentamidine	0,0032	0,0004	0,0052	0,0009

scaffold. This may partially improve solubility and penetration of studied compounds with highly lipophylic motif, taking into account the entropic effect of desorganisation of crystalline lattice caused by deplanarization of the molecules.

One should note that the presence of two negative charges of dicarboxylic acids **2a–c** is not beneficial for the trypanosomalcidal activity in the screened series of compounds which potentially can undergo deprotonation under physiologic conditions since penetration of the charged species through the lipophylic membrane of parasite is probably not favoured in this case. Spiroimide **3b** containing methoxy-substituted phenyl group possess prospective level of activity and can be used as a hit for further exploration of the SAR in these series.

For the first time 6,6-spiromaleimido-7-aryltrisubstituted thiopyrano[2,3-*d*][1,3]thiazoles were shown with activity against *Trypanosoma brucei brucei* and *Trypanosoma brucei gambiense* and, therefore, could be considered as potent hits for further antitrypanosomal drug discovery efforts.

Acknowledgment

Authors thank to Ambassy of France in Ukraine and EGIDE for fellowship for Nataliya Zelisko.

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10. Preparation 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**), *rel*-(6′*R*,7′*R*)-3′,7′-dihydro-2*H*,2′*H*,5*H*-spiro[pyrolidin-3,6′-thiopyrano[2,3-*d*]thiazol]-2,2″,5-triones (**3a–g**; **5a–c,** method **A**) and *rel*-2-[(5a*R*,11b*R*)-2,6-dioxo-3,5a,6,11b-tetrahydro-2*H*,5*H*-chromeno[4′,3′:4,5]thiopyrano[2,3-*d*]thiazol-5a-yl]acetic acid (**5**). A mixture of 10 mmol of appropriate 5-arylidene-4-thioxo-2-thiazolidinone and 11 mmol of itaconic acid or its appropriate imide was refluxed for 2 h with catalytic amount of hydroquinone (2–3 mg) for preventing of polymerization processes in 10 mL of glacial acetic acid, then left overnight at room temperature. The precipitated crystals were filtered off, washed with ethanol, and recrystallized from appropriate solvent.
11. Spectral and analytical data for compounds (**2**,**3**,**4**) are as follows. *rel-(6R,7R)-7-(4-Benzyloxy-3-methoxyphenyl)-6-carboxymethylene-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d][1,3]thiazole-6-carboxylic acid* (***2a***). Yield 69%, mp 186–189 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.82 (d, 1H, 5-H, *J* = 17.6 Hz), 2.96 (d, 1H, 5-H, *J* = 17.6 Hz), 3.19 (d, 1H, CH_2CO, *J* = 13.6 Hz), 3.35 (d, 1H, CH_2CO, *J* = 13.7 Hz), 3.72 (s, 3H, OCH_3), 3.81 (s, 1H, 7-H), 5.02 (s, 2H, OCH_2), 6.59 (d, 1H, arom., *J* = 8.0 Hz), 6.73 (s, 1H, arom.), 6.99 (d, 1H, arom., *J* = 8.4 Hz), 7.32–7.50 (m, 5H, arom.), 11.48 (s, 1H, NH), 12.62 (br.s, 2H, COOH). Anal. Calcd for $C_{23}H_{21}NO_7S_2$: C, 56.66; H, 4.34; N, 2.87. Found: C, 56.60; H, 4.20; N, 2.70. *rel-(6R,7R)-7-(4-Difluoromethoxy-3-methoxyphenyl)-6-carboxymethyl-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d][1,3]thiazole-6-carboxylic acid* (***2b***). Yield 50%, mp 210–212 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.84 (d, 1H, 5-H, *J* = 17.6 Hz), 2.97 (d, 1H, 5-H, *J* = 17.6 Hz), 3.21 (d, 1H, CH_2CO, *J* = 14.0 Hz), 3.34 (d, 1H, CH_2CO, *J* = 14.0 Hz), 3.79 (s, 3H, OCH_3), 3.79 (s, 1H, 7-H), 4.37 (s, 1H, $OCHF_2$), 6.68 (d, 1H, arom., *J* = 8.4 Hz), 6.88 (s, 1H, arom.), 7.13 (d, 1H, arom., *J* = 8.4 Hz), 11.52 (s, 1H, NH), 12.70 (br.s, 2H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.0, 26.5, 38.9, 46.8, 46.9, 56.2, 62.5, 104.3, 114.6, 117.0, 120.3, 121.1, 121.4, 138.5, 139.3, 150.4, 171.2, 172.5, 173.5. ESI-MS *m*/*z* 448 $(M+H)^+$. Anal. Calcd for $C_{17}H_{15}F_2NO_7S_2$: C, 45.64; H, 3.38; N, 3.13. Found: C, 45.50; H, 3.20; N, 3.20. *rel-(6R,7R)-7-(4-Dimethylaminophenyl)-6-carboxymethylene-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d][1,3]thiazole-6-carboxylic acid* (***2c***). Yield 61%, mp 243–245 °C (MeOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.84 (d, 1H, 5-H, *J* = 17.7 Hz), 2.87 (s, 6H, 2*CH_3), 2.95 (d, 1H, 5-H, *J* = 17.5 Hz), 3.20 (d, 1H, CH_2CO, *J* = 13.7 Hz), 3.35 (d, 1H, CH_2CO, *J* = 13.7 Hz), 3.70 (s, 1H, 7-H), 6.64 (d, 2H, arom., *J* = 8.5 Hz), 6.92 (d, 2H, arom., *J* = 8.5 Hz), 11.44 (s, 1H, NH), 12.50 (br.s, 2H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.4, 39.0, 40.5, 46.6, 47.0, 105.6, 112.4, 119.4, 127.2, 129.9, 150.1, 171.3, 172.7, 173.6. Anal. Calcd for $C_{17}H_{18}N_2O_5S_2$: C, 51.76; H, 4.60; N, 7.10. Found: C, 51.60; H, 4.50; N, 7.20. *rel-(6R,7R)-7-(4-Diethylaminophenyl)-6-carboxymethyl-2-oxo-3,5,6,7-tetrahydro-2H-thiopyrano[2,3-d][1,3]thiazole-6-carboxylic acid* (***2d***). Yield 73%, mp 224–227 °C (DMF-EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 1.06 (t, 6H, 2*CH_3CH_2), 2.83 (d, 1H, 5-H, *J* = 17.4 Hz), 2.94 (d, 1H, 5-H, *J* = 17.5 Hz), 3.16 (d, 1H, CH_2CO, *J* = 13.7 Hz), 3.29 (q, 4H, 2*CH_3CH_2), 3.35 (d, 1H, CH_2CO, *J* = 13.7 Hz), 3.67 (s, 1H, 7-H), 6.56 (d, 2H, arom., *J* = 8.5 Hz), 6.89 (d, 2H, arom., *J* = 8.5 Hz), 11.42 (s, 1H, NH), 12.52 (br.s, 2H, COOH). ^{13}C NMR (100 MHz, DMSO-d_6): 13.0, 26.5, 39.0, 44.0, 46.6, 47.0, 105.6, 111.4, 119.3, 126.0, 130.1, 147.3, 171.3, 172.7, 173.6. ESI-MS *m*/*z* 423 $(M+H)^+$. Anal. Calcd for $C_{19}H_{22}N_2O_5S_2$: C, 54.01; H, 5.25; N, 6.63. Found: C, 54.20; H, 5.10; N, 6.70. *rel-(6′R,7′R)-7′-(4-Methoxyphenyl)-1-(4-chlorophenyl)-3′,7′-dihydro-2H,2′H,5H-spiro[pyrolidin-3,6′-thiopyrano[2,3-d]thiazol]-2,2′,5-trione* (***3a***). Yield 55%, mp 240–242 °C (DMF-EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 3.06 (d, 1H, 5′-H, *J* = 16.4 Hz), 3.10 (d, 1H, 5′-H, *J* = 16.4 Hz), 3.22 (d, 1H, 4-H, *J* = 13.7 Hz), 3.37 (d, 1H, 4-H, *J* = 13.7 Hz), 3.73 (s, 3H, OCH_3), 3.82 (s, 1H, 7′-H), 6.88 (d, 2H, arom., *J* = 8.6 Hz), 7.06 (d, 2H, arom., *J* = 8.6 Hz), 7.33 (d, 2H, arom., *J* = 8.9 Hz), 7.57 (d, 2H, arom., *J* = 8.9 Hz), 11.50 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.5, 41.1, 46.6, 47.1, 55.5, 105.0, 114.1, 120.0, 120.9, 127.1, 129.1, 130.5, 131.9, 138.4, 159.1, 169.3, 171.2, 173.7. ESI-MS *m*/*z* 472 and 474 $(M+H)^+$. Anal. Calcd for $C_{22}H_{17}ClN_2O_4S_2$: C, 55.87; H, 3.62; N, 5.92. Found: C, 56.00; H, 3.50; N, 5.80. *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***). Yield

44%, mp 258–260 °C (DMF-EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.96 (d, 1H, 5′-H, J = 18.1 Hz), 3.03 (d, 1H, 5′-H, J = 18.1 Hz), 3.50 (d, 1H, 4-H, J = 12.8 Hz), 3.63 (s, 3H, OCH_3), 3.70 (d,1H, 4-H, J = 12.8 Γιι), 3.74 (s, 3H, OCH_3), 4.35 (s, 1H, 7′-H), 6.74 (br.s, 1H, arom.), 6.80 (m, 3H, arom.), 6.93 (d, 1H, arom., J = 8.5 Hz), 7.52 (d, 2H, arom., J = 8.5 Hz), 11.56 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 33.8, 36.0, 47.0, 48.2, 56.1, 56.2, 106.4, 112.1, 120.9, 128.3, 128.7, 129.4, 130.9, 133.5, 149.0, 149.5, 170.9, 173.9, 178.4. ESI-MS m/z 503 and 505 (M+H)$^+$. Anal. Calcd for $C_{23}H_{19}ClN_2O_5S_2$: C, 54.92; H, 3.81; N, 5.57. Found: C, 55.00; H, 3.70; N, 5.70. *rel-(6′R,7′R)-7′-(4-Diethylaminophenyl)-1-(4-chlorophenyl)-3′,7′-dihydro-2H,2′H,5H-spiro[pyrolidin-3,6′-thiopyrano[2,3-d]thiazol]-2,2′,5-trione* (**3c**). Yield 52%, mp 242-244 °C (DMF-EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 1.04 (t, 6H, 2*CH_3), 2.91 (d, 1H, 5′-H, J = 18.4 Hz), 2.96 (d, 1H, 5′-H, J = 18.4 Hz), 3.30 (q, 4H, 2*CH_2), 3.44 (d, 1H, 4-H, J = 12.6 Hz), 3.66 (d, 1H, 4-H, J = 12.6 Hz), 4.22 (s, 1H, 7′-H), 6.55 (d, 2H, arom., J = 8.2 Hz), 6.82 (d, 2H, arom., J = 8.4 Hz), 6.96 (br.s, 2H, arom.), 7.44 (d, 2H, arom., J = 8.4 Hz), 11.49 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 12.8, 33.6, 35.8, 41.0, 44.1, 46.7, 48.2, 107.1, 111.7, 120.5, 121.4, 128.9, 129.2, 131.1, 133.4, 147.9, 170.9, 173.9, 178.5. ESI-MS m/z 514 and 516 (M+H)$^+$. Anal. Calcd for $C_{25}H_{24}ClN_3O_3S_2$: C, 58.41; H, 4.71; N, 8.17. Found: C, 58.20; H, 4.70; N, 8.10. *rel-(6′R,7′R)-7′-(4-Dimethylaminophenyl)-1-(4-chlorophenyl)-3′,7′-dihydro-2H,2′H,5H-spiro[pyrolidin-3,6′-thiopyrano[2,3-d]thiazol]-2,2′,5-trione* (**3d**). Yield 54%, mp 240–242 °C (DMF-EtOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.86 (s, 6H, 2*CH_3), 2.92 (d, 1H, 5′-H, J = 18.5 Hz), 2.97 (d, 1H, 5′-H, J = 18.5 Hz), 3.46 (d, 1H, 4-H, J = 12.8 Hz), 3.65 (d, 1H, 4-H, J = 12.8 Hz), 4.26 (s, 1H, 7′-H), 6.62 (d, 2H, arom., J = 8.0 Hz), 6.85 (d, 2H, arom., J = 8.4 Hz), 7.01 (br.s, 2H, arom.), 7.49 (d, 2H, arom., J = 8.4 Hz), 11.51 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 33.8, 35.9, 40.6, 46.7, 48.2, 107.1, 112.4, 120.7, 122.8, 128.9, 129.3, 131.0, 133.5, 150.8, 170.9, 173.8, 178.5. ESI-MS m/z 486 and 488 (M+H)$^+$. Anal. Calcd for $C_{23}H_{20}ClN_3O_3S_2$: C, 56.84; H, 4.15; N, 8.65. Found: C, 56.70; H, 4.30; N, 8.40. *rel-2-[(5aR,11bR)-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**). Yield 45%, mp 240–242 °C (AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.60 (d, 1H, 5-H, J = 15.5 Hz), 2.72 (d, 1H, 5-H, J = 15.5 Hz), 3.40 (d, 1H, CH_2CO, J = 13.2 Hz), 3.46 (d, 1H, CH_2CO, J = 13.2 Hz), 4.41 (s, 1H, 11b-H), 7.16 (d, 1H, arom., J = 8.0 Hz), 7.26 (t, 1H, arom., J = 7.4 Hz), 7.36–7.44 (m, 2H, arom.), 11.53 (s, 1H, NH), 12.62 (br.s, 1H, COOH). ESI-MS m/z 350 (M+H)$^+$. Anal. Calcd for $C_{15}H_{11}NO_5S_2$: C, 51.57; H, 3.17; N, 4.01. Found: C, 51.70; H, 3.30; N, 3.90.

12. Preparation of *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,** method **B**). A mixture of 3 mmol of **4** and 4 mmol of appropriative aromatic amine was refluxed for 10 h in 10 mL of glacial acetic acid, and then left overnight at room temperature. The precipitated crystals were filtered off, washed with ethanol, and recrystallized from appropriate solvent.
13. Spectral and analytical data for compounds (**5**) are as follows. *rel-(6′R,7′R)-7′-(2-Hydroxyphenyl)-1-(4-chlorophenyl)-3′,7′-dihydro-2H,2′H,5H-spiro[pyrolidin-3,6′-thiopyrano[2,3-d]thiazol]-2,2′,5-trione* (**5a**). Yields 45% (method **A**), 32% (method **B**), mp 245–247 °C (MeCN). ^{1}H NMR (400 MHz, DMSO-d_6): 2.72 (d, 1H, 5′-H, J = 17.9 Hz), 3.14 (d, 1H, CH_2CO, J = 13.1 Hz), 3.24 (d, 1H, 5′-H, J = 17.9 Hz), 3.36 (d, 1H, CH_2CO, J = 13.1 Hz), 4.33 (s, 1H, 7′-H), 6.77 (m, 2H, arom.), 6.94 (d, 1H, arom., J = 7.3 Hz), 7.05 (t, 1H, arom., J = 7.7 Hz), 7.32 (d, 2H, arom., J = 8.7 Hz), 7.49 (d, 2H, arom., J = 8.7 Hz), 9.66 (s, 1H, OH), 11.44 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 28.3, 40.7, 40.9, 46.4, 104.8, 115.1, 119.5, 120.7, 125.4, 129.1, 129.2, 129.8, 131.7, 133.2, 155.7, 171.3, 173.8, 174.2, 176.3. ESI-MS m/z 459 and 461 (M+H)$^+$. Anal. Calcd for $C_{21}H_{15}ClN_2O_4S_2$: C, 54.96; H, 3.29; N, 6.10. Found: C, 55.00; H, 3.40; N, 6.20. *rel-(6′R,7′R)-7′-(2-Hydroxyphenyl)-1-(4-hydroxyphenyl)-3′,7′-dihydro-2H,2′H,5H-spiro[pyrolidin-3,6′-thiopyrano[2,3-d]thiazol]-2,2′,5-trione* (**5b**). Yields 42% (method **A**), 40% (method **B**), mp 247–250 °C (DMF-AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.68 (d, 1H, 5′-H, J = 17.9 Hz), 3.10 (d, 1H, 4-H, J = 13.1 Hz), 3.20 (d, 1H, 5′-H, J = 17.9 Hz), 3.37 (d, 1H, 4-H, J = 13.1 Hz), 4.30 (s, 1H, 7′-H), 6.76–6.81 (m, 4H, arom.), 6.94 (d, 1H, arom., J = 7.3 Hz), 7.02–7.07 (m, 4H, arom.), 9.54 (s, 1H, OH), 9.59 (s, 1H, OH), 11.40 (br.s, 1H, NH). Anal. Calcd for $C_{21}H_{16}N_2O_5S_2$: C, 57.26; H, 3.66; N, 6.36. Found: C, 57.40; H, 3.40; N, 6.30. *rel-(6′R,7′R)-7′-(2-Hydroxyphenyl)-1-(3-methyl phenyl)-3′,7′-dihydro-2H,2′H,5H-spiro[pyrolidin-3,6′-thiopyrano[2,3-d]thiazol]-2,2′,5-trione* (**5c**). Yields 45% (method **A**), 40% (method **B**), mp 247–250 °C (DMF-AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.33 (s, 3H, CH_3), 2.78 (d, 1H, 5′-H, J = 18.0 Hz), 3.21 (d, 1H, 5′-H, J = 18.0 Hz), 3.23 (d, 1H, 4-H, J = 13.0 Hz), 3.38 (d, 1H, 4-H, J = 13.0 Hz), 4.37 (s, 1H, 7′-H), 6.73-6.81 (m, 2H, arom.), 6.92 (d, 1H, arom., J = 7.5 Hz), 7.05–7.12 (m, 3H, arom.), 7.20 (d, 1H, arom., J = 7.2 Hz), 7.34 (t, 1H, arom., J = 7.8 Hz), 9.75 (s, 1H, OH), 11.59 (br.s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 21.3, 28.5, 40.7, 41.0, 46.3, 104.9, 115.1, 119.4, 120.6, 124.6, 125.5, 127.9, 129.0, 129.3, 129.8, 132.9, 138.7, 155.8, 171.2, 172.4, 174.4, 176.5. ESI-MS m/z 439 (M+H)$^+$. Anal. Calcd for $C_{22}H_{18}N_2O_4S_2$: C, 60.26; H, 4.14; N, 6.39. Found: C, 60.00; H, 4.20; N, 6.20.
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17. Drug stock solutions were prepared in pure DMSO. *T. brucei* bloodstream forms (10^5 cells/mL) were cultured in 96-well plates either in the absence or in the presence of different concentrations of inhibitors in a final volume of 200 μL. After a 72-h incubation, resazurin solution was added in each well at the final concentration of 45 μM. Fluorescence was measured at 530 nm excitation and 590 nm emission wavelengths after a further 4-h incubation. The percentage of inhibition of parasite growth rate was calculated by comparing the fluorescence of parasites maintained in the presence of drug to that of in the absence of drug. DMSO was used as control. Drugs were first tested at the concentrations of 1 and 10 μg/mL in triplicate and the experiment repeated twice. For the most active ones, IC_{50}s were determined from the dose-response curves with drug concentrations ranging from 10 μg/mL to 5 ng/mL and presented in μM. IC_{50} value is the mean +/− the standard deviation of three independent experiments.