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Anna Kryshchyshyn, Danylo Kaminsky, Igor Nektegayev, Philippe Grellier, Roman Lesyk. Isothiochromenothiazoles-A Class of Fused Thiazolidinone Derivatives with Established Anticancer Activity That Inhibits Growth of *Trypanosoma brucei*. *Scientia Pharmaceutica*, 2018, 86 (4), pp.47. 10.3390/scipharm86040047 . mnhn-02047316

HAL Id: mnhn-02047316

<https://mnhn.hal.science/mnhn-02047316>

Submitted on 24 Feb 2019

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Communication

Isothiochromenothiazoles—A Class of Fused Thiazolidinone Derivatives with Established Anticancer Activity That Inhibits Growth of *Trypanosoma brucei brucei*

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Received: 9 September 2018; Accepted: 11 October 2018; Published: 19 October 2018



Abstract: Recently, thiazolidinone derivatives have been widely studied as antiparasitic agents. Previous investigations showed that fused 4-thiazolidinone derivatives (especially thiopyranothiazoles) retain pharmacological activity of their synthetic precursors—simple 5-ene-4-thiazolidinones. A series of isothiochromeno[4a,4-d][1,3] thiazoles was investigated in an in vitro assay towards bloodstream forms of *Trypanosoma brucei brucei*. All compounds inhibited parasite growth at concentrations in the micromolar range. The established low acute toxicity of this class of compounds along with a good trypanocidal profile indicates that isothiochromenothiazole derivatives may be promising for designing new antitrypanosomal drugs.

Keywords: thiazolidinones; isothiochromenothiazoles; antitrypanosomal activity; *Trypanosoma brucei*; SAR analysis

1. Introduction

African Trypanosomiasis or sleeping sickness is one of the so-called world's neglected diseases affecting population in sub-Saharan Africa. It is caused by the parasites *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* and transmitted by the tsetse fly. Despite the fact that the number of cases reported in recent years has significantly decreased [1], nearly 60 million people remain at risk of infection. No new drugs for the African trypanosomiasis treatment have been approved since the early 1990s. The latter along with the absence of a vaccine or drugs for prophylaxis, the toxicity and the insufficient efficiency activity of the existing drugs, attest to the development of new efficient and safe antitrypanosomal agents. American trypanosomiasis or Chagas disease is caused by *Trypanosoma cruzi* transmitted by the triatomine bug. About 7 million people are infected by this parasite, mainly in Latin America. Due to human migration, the potential spreading of Chagas disease to other continents is alarming (e.g., transmission by blood transfusion). Our therapeutic arsenal against Chagas disease is furthermore very limited, with toxic drugs. So, the need for new chemotherapeutics for the American trypanosomiasis treatment is topical [2]. Moreover, it is worth mentioning that the discovery of highly active antitrypanosomals is of special interest for the search for drugs against other parasitic diseases, such as Leishmaniasis or Malaria [3,4]. This is argued by some phylogenetical similarities between protozoan parasite targets, such as dihydrofolate reductase [5] or topoisomerase I [6].

Thiazolidinone core has been widely used in medicinal chemistry as a relatively easily accessible and pharmacologically attractive scaffold [7–9]. Studies on 2,4-thiazolidinedione, rhodanine (2-thioxo-4-thiazolidinone), 2-alkyl(aryl)-substituted, and 2-R-amino(imino)-substituted 4-thiazolidinone subtypes were dedicated to the search for new antimicrobial, antidiabetic, anti-inflammatory and anticancer agents [10–12]. Recent investigations had proved 1,3-thiazoles as well as 4-thiazolidinone derivatives to be interesting and promising structures for the design of antitrypanosomal agents [2,13]. Thiazole core can be considered as a cyclic mimetic or bioisoster of the highly active anti*Trypanosoma cruzi* pharmacophore—thiosemicarbazone [14,15], which inhibits cruzain, an essential cysteine protease of *Trypanosoma cruzi* [16]. Moreover, some studies have showed that cyclization of thioureas/thiosemicarbazides into thiazole/thiazolidine derivatives is beneficial for *Trypanosoma cruzi* inhibition when compared to the antiparasitic activity of thiosemicarbazone derivatives [17]. 4-Thiazolidinone derivatives have been studied as potent trypanocidals since the 2000s, giving more active and less toxic lead compounds every year [18–22]. Our previous findings showed good antitrypanosomal activity of thiazolidinone-pyrazoline hybrids [23,24]. On the other hand, the trypanocidal effect of isothiocoumarin derivatives obtained on the base of 5-ene-4-thiazolidinones was too weak to warrant their further study [25]. Thiazolidinone derivatives, especially 5-ene-rhodanines, are often claimed as pan-assay interference compounds (PAINs) due to the conjugation of C5-exocyclic double with the C4 carbonyl group of thiazolidine core and possible Michael acceptor properties. However, this formal approach useful for large-scale screening campaigns is critically discussed in the literature [26]. In contrast, fused derivatives of 5-ene-4-thiazolidinones, namely thiopyranothiazoles retain the pharmacological profile of starting thiazolidinones and are apparently devoid of Michael acceptor functionality, constituting promising scaffolds for the development of antiparasitic agents. Earlier-established anticancer activity of isothiochromenothiazoles [27] and related thiopyranothiazoles [28–30] became one more argument for the study of their antitrypanosomal activity. Anticancer drugs such as bortezomib, aclarubicin, doxorubicin, and mitoxantrone were studied towards *Trypanosoma brucei* showing trypanocidal activities comparable to those of commercial antitrypanosomal drugs [31,32] within repurposing strategy. This gives more opportunities to find new antiparasitic compounds among already discovered agents or drugs with antiproliferative activity as well as to gain insight into the possible mechanisms of action of the latter, or even to find new biotargets essential for parasites' survival. The aim of the present work was to study whether isothiochromeno[4a,4-d][1,3]thiazoles with anticancer activity and their analogs possess antitrypanosomal activity.

2. Materials and Methods

2.1. Chemistry

All chemicals were of the analytical grade, commercially available and used without further purification. The starting compound **1** was synthesized as described previously [33]. ¹H and ¹³C NMR spectra were determined with Varian Mercury 400 (400 MHz/100 MHz) spectrometer, in DMSO-*d*₆ using tetramethylsilane as an internal standard. Elemental analyses (C, H, N) was performed at the Perkin-Elmer 2400 CHN analyzer and were within ± 0.4% from the theoretical values. LC–MS spectra were recorded on a Finnigan MAT INCOS-50 using electrospray ionization (ESI) technique. The purity of the compounds was checked by thin-layer chromatography performed with Merck Silica Gel 60 F254 aluminum sheets.

2.2. General Procedure for the Synthesis of 3-*N*-Substituted (5aR,8R,9aR)-5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2H-isothiochromeno[4a,4-d][1,3]thiazol-2-ones (**2–13**)

Ethanol solution of potassium hydroxide (0.616 g, 0.011 mol) was added to the suspension of compound **1** (2.69 g, 0.01 mol) in 30 mL ethanol; after the mixture was intensively stirred, appropriate chloroacetamide (0.011 mol) and catalytically amount of KI (0.05 g) were added. Then the mixture was

heated under reflux for 4–5 h and precipitated with water. Resulting precipitate was filtered off and recrystallized from acetonitrile (**2**, **5**, **10**, **11**), ethanol:water mixture (1:1) (**3**, **4**, **12**, **13**), toluene:hexane mixture (2:1) (**9**), isopropanol (**6**, **8**), acetic acid:water mixture (1:1) (**7**).

2.2.1. 2-[5,5,8-Trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2H-isothiochromeno[4a,4-d][1,3]thiazol-2-on-3-yl]-acetamide (**2**)

Yield 83%, mp. 128–130 °C. ^1H NMR (400 MHz, DMSO- d_6), δ : 0.89 (d, 3H, $J = 6.4$ Hz, CH_3CH), 0.98–1.03 (m, 3H), 1.29 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.48–1.60 (m, 1H), 1.68 (t, 1H, $J = 11.0$ Hz), 1.78 (d, 1H, $J = 11.0$ Hz), 1.90 (m, 2H), 2.20 (t, 1H, $J = 9.4$ Hz), 4.00 (d, 1H, $J = 17.4$ Hz, CH_2CO), 4.15 (d, 1H, $J = 17.4$ Hz, CH_2CO), 7.15 (s, 1H, NH), 7.50 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): 170.4, 167.8, 122.1, 106.0, 50.7, 48.4, 45.0, 42.4, 36.7, 34.9, 32.0, 27.6, 25.9, 23.4, 22.4. LC–MS (ESI) m/z 327 ($\text{M} + \text{H}$) $^+$. $\text{C}_{15}\text{H}_{22}\text{N}_2\text{O}_2\text{S}_2$. Calculated, % C—55.18, % H—6.79, % N—8.58; Found, % C—55.40, % H—6.90, % N—8.40.

2.2.2. *N*-(Phenyl)-2-[5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2H-isothiochromeno[4a,4-d][1,3]thiazol-2-on-3-yl]-acetamide (**3**)

Yield 93%, mp. 180–183 °C. ^1H NMR (400 MHz, DMSO- d_6), δ : 0.90–1.05 (m, 6H, CH_3CH , CH_3), 1.20 (bs, 6H, 2* CH_3), 1.45–1.55 (m, 1H), 1.65 (m, 1H), 1.75 (m, 1H), 1.90 (m, 2H), 2.34 (m, 1H), 4.30 (bs, 2H, CH_2CO), 6.90 (t, $J = 8.4$ Hz, 1H, arom.), 7.20 (t, $J = 8.4$ Hz, 2H, arom.), 7.45 (d, $J = 8.4$ Hz, 2H, arom.), 10.20 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO- d_6): 171.1, 164.7, 139.0, 129.3, 124.0, 119.5, 113.7, 106.2, 50.7, 48.7, 45.8, 42.4, 36.7, 34.9, 32.0, 27.6, 25.9, 23.4, 22.4. LCMS (ESI) m/z 401 ($\text{M} + \text{H}$) $^+$. $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_2\text{S}_2$. Calculated, % C—62.65, % H—6.51, % N—6.96; Found, % C—62.80, % H—6.80, % N—6.70.

2.2.3. *N*-(4-Methylphenyl)-2-[5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2H-isothiochromeno[4a,4-d][1,3]thiazol-2-on-3-yl]-acetamide (**4**)

Yield 75%, mp. 180–182 °C. ^1H NMR (400 MHz, DMSO- d_6), δ : 0.91 (d, 3H, $J = 6.3$ Hz, CH_3CH), 0.98–1.05 (m, 3H), 1.27 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.46–1.58 (m, 1H), 1.66 (t, 1H, $J = 11.4$ Hz), 1.76 (d, 1H, $J = 11.4$ Hz), 1.89 (m, 2H), 2.25 (s, 3H, 4- CH_3 - C_6H_4), 2.33 (t, 1H, $J = 11.1$ Hz), 4.28 (d, 1H, $J = 17.1$ Hz, CH_2CO), 4.41 (d, 1H, $J = 17.1$ Hz, CH_2CO), 7.12 (d, 2H, $J = 8.1$ Hz, 3-H, 5-H, 4-Me- C_6H_4), 7.58 (d, 2H, $J = 8.1$ Hz, 2-H, 6-H, 4-Me- C_6H_4), 10.20 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO- d_6): 170.8, 164.5, 139.0, 129.2, 124.0, 119.4, 113.6, 106.2, 50.6, 48.8, 45.6, 42.4, 36.8, 34.9, 32.1, 27.7, 25.9, 23.5, 22.4, 21.2. $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_2\text{S}_2$. Calculated, % C—63.43, % H—6.77, % N—6.72; Found, % C—63.30, % H—6.90, % N—6.80.

2.2.4. *N*-(3-Methylphenyl)-2-[5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2H-isothiochromeno[4a,4-d][1,3]thiazol-2-on-3-yl]-acetamide (**5**)

Yield 69%, mp. 184–186 °C. ^1H NMR (400 MHz, DMSO- d_6), δ : 0.93 (d, 3H, $J = 6.3$ Hz, CH_3CH), 0.99–1.08 (m, 3H), 1.29 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.52–1.63 (m, 1H), 1.67 (t, 1H, $J = 10.8$ Hz), 1.78 (d, 1H, $J = 10.8$ Hz), 1.88 (m, 2H), 2.29 (s, 3H, 3- CH_3 - C_6H_4), 2.35 (t, 1H, $J = 8.1$ Hz), 4.30 (d, 1H, $J = 17.1$ Hz, CH_2CO), 4.43 (d, 1H, $J = 17.1$ Hz, CH_2CO), 6.90 (d, 1H, $J = 7.5$ Hz, 4-H, 3- CH_3 - C_6H_4), 7.21 (t, 1H, $J = 7.8$ Hz, 5-H, 3- CH_3 - C_6H_4), 7.33 (d, 1H, $J = 8.1$ Hz, 6-H, 3-Me- C_6H_4), 7.43 (s, 1H, 2-H, 3-Me- C_6H_4), 10.23 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO- d_6): 170.5, 164.9, 152.7, 149.5, 138.9, 136.5, 129.1, 120.0, 114.7, 108.3, 50.7, 48.6, 47.0, 42.4, 36.7, 34.9, 32.0, 27.6, 25.9, 23.4, 22.4, 21.6. $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_2\text{S}_2$. Calculated, % C—63.43, % H—6.77, % N—6.72; Found, % C—63.60, % H—6.80, % N—6.90.

2.2.5. *N*-(4-Isopropylphenyl)-2-[5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2H-isothiochromeno[4a,4-d][1,3]thiazol-2-on-3-yl]-acetamide (**6**)

Yield 68%, mp. 241–244 °C. ^1H NMR (400 MHz, DMSO- d_6), δ : 0.90 (d, $J = 6.8$ Hz, 3H, CH_3CH), 1.00–1.05 (m, 3H), 1.16 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.29 (d, $J = 12.9$ Hz, 2* CH_3 , 6H), 1.45–1.55 (m, 1H), 1.65 (t, $J = 12.0$ Hz, 1H), 1.75 (d, $J = 12.0$ Hz, 1H), 1.90 (m, 2H), 2.34 (t, $J = 8.0$ Hz, 1H), 2.85 (m, 1H,

CHMe₂), 4.28 (d, *J* = 18.0 Hz, 1H, CH₂CO), 4.38 (d, *J* = 18.0 Hz, 1H, CH₂CO), 7.18 (d, *J* = 8.4 Hz, 2H, arom.), 7.45 (d, *J* = 8.4 Hz, 2H, arom.), 10.19 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): 171.3, 164.0, 152.5, 150.0, 139.0, 136.3, 129.5, 119.1, 107.7, 50.6, 47.6, 42.8, 36.8, 35.0, 34.9, 32.2, 27.9, 26.1, 23.8, 23.6, 22.6. C₂₄H₃₂N₂O₂S₂. Calculated, % C—64.83, % H—7.25, % N—6.30; Found, % C—64.60, % H—7.10, % N—6.40.

2.2.6. *N*-(4-Sulfonamido-phenyl)-2-[5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2*H*-isothiochromeno[4a,4-*d*][1,3]thiazol-2-on-3-yl]-acetamide (7)

Yield 79%, mp. 111–114 °C. ¹H NMR (400 MHz, DMSO-*d*₆), δ: 0.88–0.92 (m, 3H, CH₃CH); 0.97–1.03 (m, 3H), 1.27–1.32 (m, 6H, 2*CH₃), 1.49–1.57 (m, 1H), 1.61–1.67 (m, 1H), 1.71–1.81 (m, 1H), 1.86–1.89 (m, 1H), 2.27–2.34 (m, 1H), 4.35 (d, *J* = 17.2 Hz, 1H, CH₂CO), 4.43 (d, *J* = 17.0 Hz, 1H, CH₂CO), 7.24 (s, 2H, NH₂), 7.71 (d, 2H, *J* = 8.7 Hz, 2H, C₆H₄), 7.77 (d, 2H, *J* = 8.4 Hz, 2H, C₆H₄), 10.61 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): 170.5, 165.4, 141.8, 139.2, 127.3, 122.0, 119.1, 106.3, 50.4, 48.7, 45.9, 42.6, 36.6, 34.8, 32.0, 27.7, 25.8, 23.6, 22.4. C₂₁H₂₇N₃O₄S₃. Calculated, % C—52.37, % H—5.65, % N—8.72; Found, % C—52.50, % H—5.80, % N—8.60.

2.2.7. *N*-(4-Acetyl-amino-phenyl)-2-[5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2*H*-isothiochromeno[4a,4-*d*][1,3]thiazol-2-on-3-yl]-acetamide (8)

Yield 87%, mp. 259–263 °C. ¹H NMR (400 MHz, DMSO-*d*₆), δ: 0.94 (d, *J* = 6.8 Hz, 3H, CH₃CH), 1.00–1.18 (m, 3H), 1.28 (s, 3H, CH₃), 1.32 (s, 3H, CH₃), 1.45–1.55 (m, 1H), 1.66 (m, 1H), 1.75 (m, 1H), 1.88 (m, 2H), 2.02 (s, 3H, CH₃CO), 2.33 (m, 1H), 4.30 (d, *J* = 18.0 Hz, 1H, CH₂CO), 4.40 (d, *J* = 18.0 Hz, 1H, CH₂CO), 7.50 (bs, 4H, arom.), 9.91 (s, 1H, NH), 10.24 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): 170.5, 168.4, 164.3, 135.6, 134.2, 122.1, 119.9, 106.2, 50.7, 48.6, 45.7, 42.4, 36.7, 34.9, 32.0, 27.6, 25.9, 24.3, 23.4, 22.3. LCMS (ESI) *m/z* 460 (M + H)⁺. C₂₃H₂₉N₃O₃S₂. Calculated, % C—60.10, % H—6.36, % N—9.14; Found, % C—60.00, % H—6.60, % N—8.90.

2.2.8. 4-[2-(5,5,8-Trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2*H*-isothiochromeno[4a,4-*d*][1,3]thiazol-2-on-3-yl)-acetyl-amino]-benzoic acid ethyl ester (9)

Yield 75%, mp. 196–198 °C. ¹H NMR (400 MHz, DMSO-*d*₆), δ: 0.93 (d, 3H, *J* = 6.4 Hz, CH₃CH), 1.00–1.10 (m, 3H), 1.28 (s, 3H, CH₃), 1.32 (s, 3H, CH₃), 1.34 (t, 3H, CH₃CH₂O), 1.48–1.60 (m, 1H), 1.67 (t, 1H, *J* = 11.2 Hz), 1.80 (d, 1H, *J* = 11.2 Hz), 1.90 (m, 2H), 2.33 (t, 1H, *J* = 9.0 Hz), 4.48 (q, 2H, CH₃CH₂O), 4.30 (d, 1H, *J* = 17.1 Hz, CH₂CO), 4.42 (d, 1H, *J* = 17.1 Hz, CH₂CO), 7.67 (d, 2H, *J* = 8.0 Hz, 3-H, 5-H, 4-COOEt-C₆H₄), 7.90 (d, 2H, *J* = 8.0 Hz, 2-H, 6-H, 4-COOEt-C₆H₄), 10.53 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): 170.6, 165.7, 165.4, 143.3, 130.9, 125.1, 122.1, 119.0, 106.4, 61.0, 50.8, 48.8, 45.9, 42.4, 36.8, 34.9, 32.0, 27.6, 25.9, 23.5, 22.4, 14.7. C₂₄H₃₀N₂O₄S₂. Calculated, % C—60.73, % H—6.37, % N—5.90; Found, % C—60.50, % H—6.50, % N—5.70.

2.2.9. *N*-(4-Chlorophenyl)-2-[5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2*H*-isothiochromeno[4a,4-*d*][1,3]thiazol-2-on-3-yl]-acetamide (10)

Yield 78%, mp. 206–208 °C. ¹H NMR (400 MHz, DMSO-*d*₆), δ: 0.86 (d, *J* = 7.0 Hz, 3H, CH₃CH), 0.91–1.03 (m, 3H), 1.23 (s, 3H, CH₃), 1.27 (s, 3H, CH₃), 1.40–1.49 (m, 1H), 1.54 (m, 1H), 1.71 (d, 1H, *J* = 11.7 Hz), 1.80 (m, 2H), 2.27 (t, 1H, *J* = 7.8 Hz), 4.32 (d, 1H, *J* = 16.6 Hz, CH₂CO), 4.48 (d, 1H, *J* = 16.7 Hz, CH₂CO), 7.67 (d, 2H, *J* = 8.2 Hz, arom.), 7.80 (d, 2H, *J* = 8.1 Hz, arom.), 10.72 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): 170.8, 164.9, 138.7, 129.3, 124.3, 119.0, 113.5, 106.9, 50.6, 47.8, 42.7, 36.8, 35.1, 32.0, 27.6, 26.2, 25.0, 22.4. C₂₁H₂₅ClN₂O₂S₂. Calculated, % C—57.72, % H—5.77, % N—6.41; Found, % C—57.50, % H—5.90, % N—6.60.

2.2.10. *N*-(3,4-Dichlorophenyl)-2-{5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2*H*-isothiochromeno [4a,4-*d*][1,3]thiazol-2-on-3-yl}-acetamide (**11**)

Yield 67%, mp. 210–212 °C. ¹H NMR (400 MHz, DMSO-*d*₆), δ: 0.89 (d, *J* = 7.3 Hz, 3H, CH₃CH), 0.89–0.95 (m, 3H), 1.26 (s, 3H, CH₃), 1.32 (s, 3H, CH₃), 1.42–1.50 (m, 1H), 1.60 (m, 1H), 1.72–1.76 (m, 1H), 1.86–1.90 (m, 2H), 2.31 (t, 1H, *J* = 8.0 Hz), 4.37 (d, 1H, *J* = 17.1 Hz, CH₂CO), 4.52 (d, 1H, *J* = 17.3 Hz, CH₂CO), 7.71 (d, 1H, *J* = 8.3 Hz, arom.), 7.92 (d, 1H, *J* = 8.3 Hz, arom.), 8.01 (s, 1H, arom), 10.94 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): 170.5, 165.4, 139.0, 131.6, 131.3, 125.5, 122.0, 120.8, 119.6, 106.4, 50.8, 48.7, 45.8, 42.4, 36.7, 34.9, 32.0, 27.6, 25.9, 23.4, 22.4. C₂₁H₂₄Cl₂N₂O₂S₂. Calculated, % C—53.50, % H—5.13, % N—5.94; Found, % C—53.70, % H—5.20, % N—6.00.

2.2.11. *N*-(2-Trifluoromethylphenyl)-2-{5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2*H*-isothiochromeno [4a,4-*d*][1,3]thiazol-2-on-3-yl}-acetamide (**12**)

Yield 91%, mp. 163–165 °C. ¹H NMR (400 MHz, DMSO-*d*₆), δ: 0.90 (d, 3H, *J* = 6.6 Hz, CH₃CH), 0.95–1.08 (m, 3H), 1.27 (s, 3H, CH₃), 1.34 (s, 3H, CH₃), 1.45–1.60 (m, 1H), 1.67 (t, 1H, *J* = 11.3 Hz), 1.75 (d, 1H, *J* = 11.3 Hz), 1.90 (m, 2H), 2.32 (t, 1H, *J* = 9.0 Hz), 4.36 (d, 1H, *J* = 17.4 Hz, CH₂CO), 4.47 (d, 1H, *J* = 17.4 Hz, CH₂CO), 7.47 (t, 1H, *J* = 7.8 Hz, 5-H, 2-CF₃-C₆H₄), 7.49 (d, 1H, *J* = 7.8 Hz, 3-H, 2-CF₃-C₆H₄), 7.70 (t, 1H, *J* = 7.5 Hz, 4-H, 2-CF₃-C₆H₄), 7.75 (d, 1H, *J* = 7.5 Hz, 6-H, 2-CF₃-C₆H₄), 9.93 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): 170.5, 166.0, 135.0, 133.6, 130.1, 127.3, 126.8 (q, *J* = 185 Hz), 125.0, 123.0 (q, *J* = 19.8 Hz), 122.0, 106.3, 50.8, 48.5, 45.3, 42.4, 36.8, 34.9, 32.0, 27.6, 25.9, 23.4, 22.4. LCMS (ESI) *m/z* 471 (M + H)⁺. C₂₂H₂₅F₃N₂O₂S₂. Calculated, % C—56.15, % H—5.35, % N—5.95; Found, % C—56.30, % H—5.60, % N—6.10.

2.2.12. *N*-(3-Trifluoromethylphenyl)-2-{5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2*H*-isothiochromeno [4a,4-*d*][1,3]thiazol-2-on-3-yl}-acetamide (**13**)

Yield 80%, mp. 182–184 °C. ¹H NMR (400 MHz, DMSO-*d*₆), δ: 0.92 (d, 3H, *J* = 6.2 Hz, CH₃CH), 0.98–1.06 (m, 3H), 1.28 (s, 3H, CH₃), 1.32 (s, 3H, CH₃), 1.51–1.56 (m, 1H), 1.67 (t, 1H, *J* = 11.3 Hz), 1.75 (d, 1H, *J* = 11.3 Hz), 1.88 (m, 2H), 2.34 (m, 1H), 4.37 (d, 1H, *J* = 17.4 Hz, CH₂CO), 4.47 (d, 1H, *J* = 17.1 Hz, CH₂CO), 7.43 (d, 1H, *J* = 7.3 Hz, 3-CF₃-C₆H₄), 7.59 (d, 1H, *J* = 7.1 Hz, 2-CF₃-C₆H₄), 7.72–7.74 (m, 1H, 3-CF₃-C₆H₄), 8.09 (s, 1H, 3-CF₃-C₆H₄), 10.74 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): 170.6, 165.5, 139.8, 130.7 (q, *J* = 20 Hz), 123.1 (q, *J* = 160 Hz), 122.0, 120.5, 114.6 (q, *J* = 5 Hz), 111.8, 109.3, 103.4, 50.8, 48.8, 44.0, 42.6, 36.8, 34.8, 32.0, 27.6, 25.9, 23.5, 22.4. LCMS (ESI) *m/z* 471 (M + H)⁺. C₂₂H₂₅F₃N₂O₂S₂. Calculated, % C—56.15, % H—5.35, % N—5.95; Found, % C—56.00, % H—5.50, % N—5.70.

2.3. Pharmacology

2.3.1. Antitrypanosomal Activity Assay

Bloodstream forms of *Trypanosoma brucei* strain 90-13 were cultured in HMI9 medium supplemented with 10% FCS (fetal calf serum) at 37 °C under an atmosphere of 5% CO₂ [34]. In all experiments, log-phase parasite 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 previously described [35,36]. Drug stock solutions were prepared in pure DMSO. *Trypanosoma brucei* bloodstream forms (10³ cells/well) 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 72 h incubation, resazurin solution was added in each well at the final concentration of 45 μM and fluorescence was measured at 530 nm and 590 nm absorbance 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. Concentration inhibiting 50% of parasite growth (IC₅₀) was determined from the dose–response curve with drug concentrations ranging from 10 to 0.625 μg/mL and presented in μM.

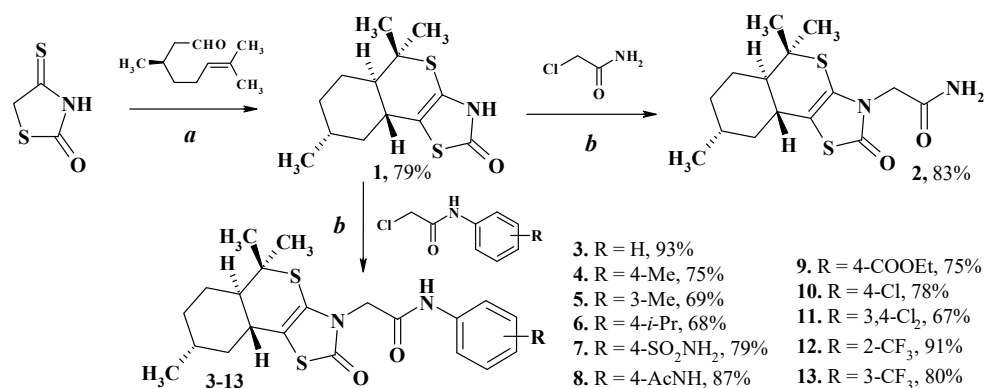
2.3.2. Acute Toxicity In Vivo

The experiments were conducted on white male mice weighing 23–25 g. Compounds were dissolved in saline solution (0.9% NaCl) with 1–2 drops of Polysorbate 80 (Tween-80®). After dissolution, they were administered to mice via intraperitoneal route. The LD₅₀ was evaluated for 4 or 5 different doses each on 6 animals and calculated by the Litchfield and Wilcoxon method [37].

3. Results and Discussion

3.1. Chemistry

Isothiochromeno[4a,4-d][1,3]thiazoles **1–13** were synthesized (compounds **2**, **4**, **5**, **9**, **11**, **13**-resynthesized) according to the known synthetic protocol [27,38]. Starting (5a*R*,8*R*,9a*R*)-5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-octahydro-2*H*-isothiochromeno[4a,4-d][1,3]thiazole-2-one **1** was obtained in the Knoevenagel-*hetero*-Diels–Alder reaction of 3,7-dimethyloct-6-enal (citronellal) and 4-thioxo-2-thiazolidinone (isorhodanine) in the presence of ethylenediamine diacetate in acetonitrile medium at room temperature [33]. Target compounds **2–13** were synthesized in the alkylation reactions of **1** potassium salt by different chloroacetamides in ethanol. The reaction goes in situ through the stage of potassium salt formation which was not isolated (Scheme 1).



Scheme 1. Target isothiochromeno[4a,4-d][1,3]thiazoles synthesis. Reagents and conditions: (a) isorhodanine (1.0 equiv.), EDDA (0.15 equiv.), 3,7-dimethyloct-6-enal (1.3 equiv.), MeCN, rt, 24 h, 79% [33]; (b) appropriate chloroacetamide (1.1 equiv.), KOH (1.1 equiv.), KI (cat. amount), EtOH, reflux, 4–5 h, 67–93%.

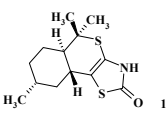
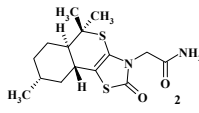
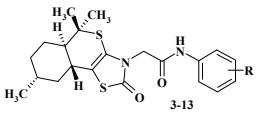
Structure and purity of compounds were confirmed by NMR spectroscopy and chromatography data (experimental part). Stereoconfiguration of compounds were assigned based on our previous data (spectral data, X-ray analysis) [33].

3.2. Pharmacology

Synthesized isothiochromeno[4a,4-d][1,3]thiazoles inhibited the growth of *Trypanosoma brucei* bloodstream forms with IC₅₀ values in the micromolar range (Table 1) comparable to Nifutimox (IC₅₀ = 2.39 μM). Increasing activity from the starting compound **1** (IC₅₀ = 29.3 μM) as well as **2** (IC₅₀ = 44.4 μM) proves the necessity of aryl substituents in the N3 position of the main core for the trypanocidal activity. Substituents in the aryl fragment also influence the antitrypanosomal activity. For example, halogen atoms in the aryl moiety significantly increase the inhibition activity of derivatives **10–13** (IC₅₀ ranging from 2.97 to 8.50 μM). Interestingly, previously tested and structurally related thiopyranothiazoles with a norbornane moiety and the same fragments in the N3 position of the thiazolidine core [29] showed the same levels of IC₅₀ values against *Trypanosoma brucei*. Probably, an acetamide moiety with different substituents in the aryl ring has an impact on the spatial arrangement of atoms that increases

the molecules affinity to the yet unknown biotarget. On the other hand, an ester moiety in the aryl ring of **9** also highly contributes to the trypanocidal activity ($IC_{50} = 1.55 \mu M$).

Table 1. Antitrypanosomal and anticancer activity of target isothiochromeno[4a,4-d]thiazol-2-ones.

Compound	R	<i>Trypanosoma brucei</i> IC_{50} (μM)	Anticancer Activity DTP 60 Lines Assay ^{1,2} [27]	Acute Toxicity LD_{50} (mg/kg)
				
1	-	29.32		240
2	-	44.41	10 μM assay: mean GP—106%; range of growth—78.80–144.06%; EKVX (non-small-cell lung cancer)—78.80% ¹	
3	H	10.18		
4	4-Me	10.80	10 μM assay: mean GP—85.70%; range of growth—5.23–119.66%; UACC (melanoma)—5.23%, OVCAR-8 (ovarian cancer)—34.80%, MCF-7 (breast cancer)—52.50%, NCI-H522 (non-small-cell lung cancer)—58.10	
5	3-Me	10.08	0.1–100 μM assay: GI_{50} : CCRF-CEM (leukemia)—1.62 μM , MOLT-4 (leukemia)—5.94 μM , RPMI-8226 (leukemia)—5.69 μM , SR (leukemia)—12.1 μM , HOP-92 (non-small-cell lung cancer)—2.13 μM , NCI-H460 (non-small-cell lung cancer)—79.6 μM , SK-MEL-5 (melanoma)—53.9 μM , PC-3 (prostate cancer)—7.43 μM ²	
6	4- <i>i</i> -Pr	14.17		
7	4-SO ₂ NH ₂	14.12		
8	4-AcNH	18.28		
9	4-COOEt	1.55		320
10	4-Cl	5.27		480
11	3,4-Cl ₂	4.67	10 μM assay: mean GP—85.89%; range of growth—46.28–111.66%; HOP-92 (non-small-cell lung cancer)—46.90%, HS 578T (breast cancer)—46.28%, RPMI-8226 (leukemia)—46.87%, MOLT-4 (leukemia)—58.86%	
12	2-CF ₃	8.50	10 μM assay: mean GP—84.74%; range of growth—0.76–116.02%; MOLT-4 (leukemia)—36.02%, OVCAR-8 (ovarian cancer)—28.87, UACC-257 (melanoma)—0.76%, MCF-7 (breast cancer)—56.32%	
13	3-CF ₃	2.97		270

¹ Anticancer screening data in one dose (10 μM). Activity is reported as the growth percent (GP) of treated cells compared to untreated control cells. Most sensitive cell lines are indicated. ² Anticancer screening data at concentrations ranging from 0.1 to 100 μM . GI_{50} —molar concentration of the compound that inhibits 50% net cell growth. Most sensitive cell lines are indicated.

Establishment of any anticancer and antitrypanosomal activity correlation for isothiochromenothiazoles was also an issue of the study. Previously, some of the synthesized isothiochromenothiazoles were studied for their anticancer activity within a Developmental Therapeutic Program protocol (National Cancer Institute, Bethesda, MD, USA) [27]. Among isothiochromeno[4a,4-d]thiazoles tested at a concentration of 10^{-5} M, selectivity towards melanoma cell line UACC-257 was observed for *N*-(2-trifluoromethylphenyl)-2-[5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-oktahydro-2*H*-isothiochromeno[4a,4-d]thiazol-2-on-3-yl]-acetamide **12** (GP = 0.76%) and *N*-(*p*-tolyl)-2-[5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-oktahydro-2*H*-isothiochromeno[4a,4-d]thiazol-2-on-3-yl]-acetamide **4** (GP = 5.23%). These two derivatives also inhibited growth of more than 60% of cells of ovarian cancer line OVCAR-8. Change of the trifluoromethyl moiety in the benzene ring to 3,4-dichlorobenzene did not contribute to anticancer activity which was rather low. Worth mentioning is that the absence of arylidene ring in the acetamide substituent in the N3-position significantly decreased the antitumor

potential as well as the antiparasitic activity. This attests to the crucial role of aryl substituents in the N3 position of the isothiochromeno[4a,4-d]thiazole core for the antiproliferative activity. *N*-(3-Methylphenyl)-2-{5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-oktahydro-2*H*-isothiochromeno[4a,4-d]thiazol-2-on-3-yl}-acetamide **5** was tested in a range of concentrations from 0.1 to 100 μ M and showed the highest growth inhibitory levels (GI_{50}) against cell lines of leukemia, non-small-cell lung cancer and prostate cancer [27]. One more argument in favor of a dual action for the tested derivatives is a significant decrease of trypanocidal potential and the absence of anticancer activity (inhibition of cancer cells growth less than by 20%) for *N*-(5,5,8-trimethyl-3,5,5a,6,7,8,9,9a-oktahydro-2*H*-isothiochromeno[4a,4-d]thiazol-2-on-3-yl)-acetamide **2**.

For derivatives with the highest trypanocidal activity, **9**, **10**, and **13** and the starting compound **1**, the acute toxicity (mice) was studied and their LD_{50} were determined [35,36]. The stock solutions of the compounds used in this study were prepared immediately before usage and increasing amounts of substances (100–1000 mg/kg) were injected intraperitoneally. The LD_{50} values were calculated according to Litchfield and Wilcoxon. These derivatives show a little acute toxicity in mice with LD_{50} values within the range of 240–480 mg/kg.

4. Conclusions

A series of isothiochromeno[4a,4-d][1,3]thiazole derivatives was synthesized and investigated in an in vitro assay against *Trypanosoma brucei brucei*. Compounds showed inhibitory activity towards the parasite growth at concentrations in the micromolar range. Structure–activity relationships reveal the positive influence of N3-substituent for the trypanocidal activity. The same trend was found for the anticancer activity of compounds. This is an additional argument in favor of the search for compounds with dual action (antitrypanosomal and anticancer). Along with this, low acute toxicity of compounds indicates that isothiochromenothiazoles may be used in further directed design of antitrypanosomal agents.

Author Contributions: R.L., A.K., and P.G. conceived and designed the project and paper; D.K. and A.K. performed the synthesis of target compounds; P.G. tested compounds for antitrypanosomal activity; I.N. studied acute toxicity of compounds in vivo; R.L. and A.K. wrote the paper. All authors read and approved the final manuscript.

Funding: The project was partly supported by the State Fund for Fundamental Research of Ukraine (F76/72-2017, F76/13-2018) and the Ministry of Education and Science of Ukraine (M/181-2017; 92-2018), Ukrainian-France program “Dnipro” M/188-2015; M/71-2016).

Conflicts of Interest: The authors declare no conflict of interest.

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