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Synthesis of 5-aryl/heterylidene substituted 2-imino-4-thiazolidinones possessing 1,3,4-thiadiazole moiety and their antitrypanosomal activity

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A B S T R A C T

A novel 1,3,4-thiadiazole containing 2-iminothiazolidine-4-ones **4a-b** were synthesized through the reaction of 2-chloro-*N*-(5-ethyl/allylsulfanyl-[1,3,4]thiadiazol-2-yl)-acetamides **1a-b** with ammonium thiocyanate in dry acetone. Condensation of **4a-b** with various carbonyl compounds according to the standard Knoevenagel procedure yielded the corresponding 5-arylidene- (**5a-d**), 5-heterylidene- (**6a-c**), 5-isatinylidene- (**7a-b**) and 5-(3-phenyl-2-propene-1-ylidene)- (**8a-b**) derivatives. All the newly synthesized compounds were confirmed by their elemental analysis and spectral data. Synthesized compounds **4a**, **5a**, **5b**, **6a**, **6c** and **7a** were screened for their *in vitro* antitrypanosomal activity against *T. brucei gambience* (Feo strain). The 5-ylidene substituted compounds with *S*-allyl group in position 5 of thiadiazole cycle (**5a**, **5b**, **6a** and **7a**) displayed good to excellent antitrypanosomal potency with a range IC_{50} = 7.3-12.8 μM.

1. Introduction

Synthesis, reactions and pharmacological properties of substituted 4-thiazolidinones constitute a significant part of modern medicinal chemistry. Compounds possessing 4-thiazolidinone core have been found to exhibit a number of biological activities including anticancer[1,2], antibacterial[3,4], antiviral[5,6], antifungal[7], trypanocidal[8], analgesic[9], anti-inflammatory[10] action etc. On the other hand, 1,3,4-thiadiazole derivatives continue to attract the interest of chemists and microbiologists due to their remarkable pharmacological profile, among which antiproliferative[11], antiviral[12], antimicrobial[13], anticonvulsant[14], telomerase[15] and aminopeptidase[16] inhibition, CNS depressant[17] activities are the most familiar. Among these, few are well known as commercial products such as *Cefazedone* and *Cefazolin* (first generation semi-synthetic cephalosporins), *Lucosir* and *Globucid* (antimicrobial), *Acetazolamide* and *Methazolamide* (carbonic anhydrase inhibitors), *Megazol* (antimicrobial and trypanocidal)[18] are being sold in the market.

Interesting and perspective approach for drug-like molecules build-up is based on the conception of molecular hybridization approach, which includes a conjugation of several pharmacologically attractive scaffolds, in particular 4-thiazolidinone and 1,3,4-thiadiazole. Thus, thiazolidinone-thiadiazole conjugates have been reported to be effective antibacterial[19,20], antiviral[21,22], anticancer[23], antioxidant[23,24] and dual COX-2/15-LOX inhibition[25] agents. Based on the above reports we tried to conjugate thiazolidinone and 1,3,4-thiadiazole motifs under one construct to develop potential

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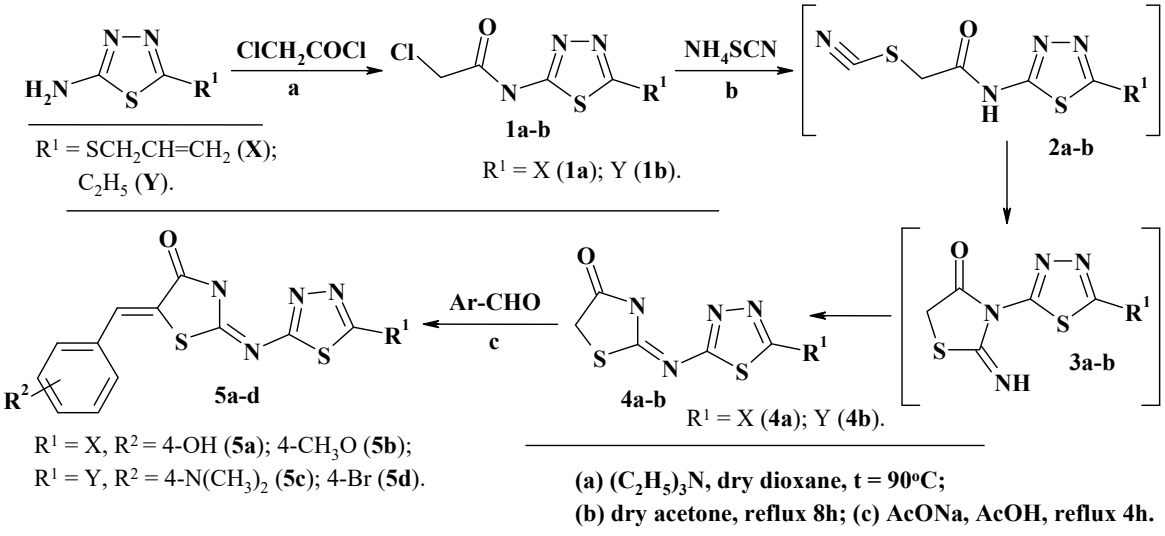
trypanocidal agents. The present work describes the synthesis of a new 2-(5-ethyl/allylsulfanyl-1,3,4-thiadiazole-2-ylimino)thiazolidin-4-ones and their 5-ylidene derivatives and investigation for their antitrypanosomal activity *in vitro*.

2. Results and discussion

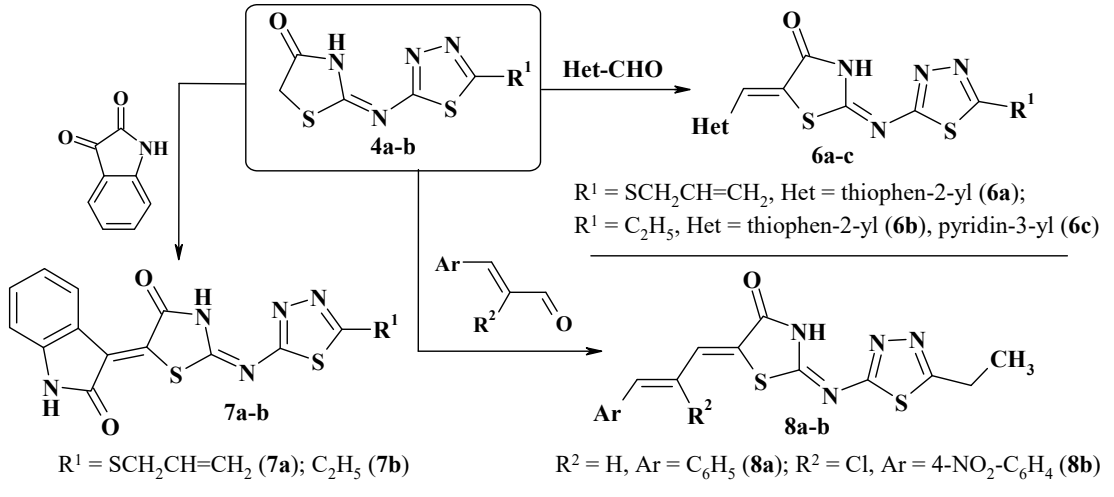
2.1. Chemistry

Based on the reaction of 5-ethyl- or 5-allylsulfanyl-1,3,4-thiadiazole-2-amine with chloroacetyl chloride in dioxane medium, the corresponding 2-chloro-*N*-(5-ethyl/allylsulfanyl-[1,3,4]thiadiazol-2-yl)-acetamides **1a-b** have been obtained. An effective and convenient approach based on the interaction of *N*-substituted 2-chloroacetamides with thiocyanates was proposed for the further synthesis of non-condensed 2-iminothiazolidin-4-one derivatives. This transformations through a series of intermediates **2a-b** and **3a-b** lead to the formation of titled 2-iminothiazolidine-4-ones containing 1,3,4-thiadiazole moiety **4a-b** and were similar to described by us previously oxadiazolyl-thiazolidinones.[26] Further the obtained compound **4a-b** were reacted with aromatic aldehydes yielded a group of 5-arylidene derivatives **5a-d** (**Scheme 1**).



Scheme 1. Synthesis of 2-(1,3,4-thiadiazole-2-ylimino)thiazolidin-4-ones and their 5-arylidene derivatives

The obtained thiadiazolyl-iminothiazolidinones **4a-b** also were modified by interaction with thiophene-2-carbaldehyde, pyridine-3-carbaldehyde, isatine or cinnamaldehyde derivatives according to the standard Knoevenagel condensation procedure (medium – acetic acid, catalyst – fused sodium acetate). As a result of these transformations the synthesis of target 5-heterylidene-, 5-isatinylidene- and 5-(3-phenyl-2-propene-1-ylidene)- substituted 2-(1,3,4-thiadiazol-2-ylimino)thiazolidin-4-ones **6a-c**, **7a-b** and **8a-b** was performed according to the **Scheme 2**.



Scheme 2. Synthesis of 5-heterylidene-, 5-isatinylidene- and 5-(3-phenyl-2-propene-1-ylidene)- substituted 2-(1,3,4-thiadiazole-2-ylimino)thiazolidin-4-ones

Structures of the synthesized compounds were confirmed by ^{1}H NMR spectroscopy and elemental analysis. The obtained spectroscopic data for all new compounds are in accordance with the proposed structures. In particular, the protons of the cyclic methylene group in the ^{1}H NMR spectra of the 5-unsubstituted derivatives **4a-b** appear as a singlet at δ ~ 4.09-4.11 ppm, which reliably confirms the passage of cyclization. The signal of a 5-methylidene proton –CH= for the target 5-aryl/heterylidene compounds **5a-d** and **6a-c** resonates as a singlet with a higher chemical shift in the range of δ ~ 7.64-7.75 ppm confirming a *Z*-configuration of the exocyclic C=C bond at the 5-arylidene fragment. [27-29]

For 5-unsubstituted isatin system (compounds **7a-b)** the characteristic pattern from two doublets (δ ~ 6.91-6.93 and δ ~ 8.81-8.82 ppm, respectively) and two triplets (δ ~ 7.05-7.07 and δ ~ 7.36-7.38 ppm) have been observed. It is important to note that the proton signal in position 4 of the isatin fragment is significantly shifted to the region of a weak magnetic field, which can be explained by the influence of the carbonyl group in position 4 of the thiazolidine ring and, accordingly, the formation of the *Z*-isomer. The signal of NH proton in position 1 of the isatin fragment appears as a singlet in the range of δ ~ 11.21-11.22 ppm.

The synthesized 2-thiadiazolylimino-4-thiazolidinones **4a-b** and their 5-ylidene derivatives **5a-d**, **6a-c**, **7a-b** and **8a-b** are characterized by prototropic amino-imino tautomerism. Considering that the chemical shift value of the NH-proton signal is shifted towards the weak magnetic field (δ ~ 12.28-12.33 ppm for **4a-b** or δ ~ 12.60-13.10 ppm for **5a-d**, **6a-c**, **7a-b** and **8a-b**), which is more characteristic to the endocyclic lactam proton than the exocyclic imino one.[30,31] It suggests that the obtained 1,3,4-thiadiazole substituted 4-thiazolidinones in the dissolved state have exclusively an imino form, which is consistent with previously obtained data for structurally related oxadiazole based derivatives.[26]

2.2. In vitro evaluation of antitrypanosomal activity

Six of synthesized compounds (**4a**, **5a**, **5b**, **6a**, **6c** and **7a)** were screened for inhibitory activity against *T. brucei gambience* (Feo strain). Compounds were first tested at fixed concentrations of 50, 10 and 1 mg/ml. As a result, the value of percentage inhibition of parasite growth was determined by the level of *Alamar Blue* fluorescence in comparison with the control. IC_{50} values were further determined for those showing significant trypanocidal activity at 10 mg/ml (>40-50% of parasite growth inhibition) (Table 1). Nifurtimox, which is used in combination with eflornithine for the gambiense human African trypanosomiasis therapy[32], was selected as a reference antiparasitic drug.

Table 1. Antitrypanosomal activity of synthesized 1,3,4-thiadiazole substituted 2-iminothiazolidin-4-ones and their 5-ylidene derivatives.

Comp	Ar[1]	Ar(Het)	IC_{50}		IC_{50}	
			µg/ml	SD	µM	SD
4a (2b)	$SCH_2CH=CH_2$	–	> 10	–	> 36,7	–
5a (3c)	$SCH_2CH=CH_2$	4-OH-C_6H_4	2.8	0.4	7.3	0.9
5b (3d)	$SCH_2CH=CH_2$	4-CH_3O-C_6H_4	3.9	0.3	10.1	0.6
6a (4b)	$SCH_2CH=CH_2$	thiophen-2-yl	4.2	0.2	11.4	0.6
6c (4c)	C_2H_5	pyridine-3-yl	> 10	–	> 31.5	–
7a (5b)	$SCH_2CH=CH_2$	2-oxoindol-3-ylidene	5.1	1.0	12.8	2.5
Nifurtimox	–	–	–	–	4.4	0.7

The obtained results showed that among the tested 1,3,4-thiadiazole-substituted 5-ylidene-2-imino-4-thiazolidinones, four compound namely **5a**, **5b**, **6a** and **7a** have a good to excellent trypanocidal effect with a range of IC_{50} values = 7.3÷12.8 µM, which was slightly lower than that of the reference drug nifurtimox (IC_{50} = 4.4 µM). Compounds **4a** and **6c** did not show an inhibitory effect on the growth of parasites in the tested conditions. In addition, it was found that the determining impact for increase of the antitrypanosomal effect is the presence of 5-ylidene substituent in position 5 of thiazolidinone core (except compound **4a**) and *S*-allyl group in position 5 of thiadiazole cycle (except **6c**).

In general, this study gives great evidence for the different uses of organic and inorganic compounds in the search for potential pharmacologically active compounds, as reported before. In particular, various 4-thiazolidinone derivatives have been reported to possess promising chemotherapeutic properties including antitrypanosomal activity[8,33,34]. It was found that the trypanocidal action of substituted 4-thiazolidinones can be expressed through inhibition of different biotargets such as cysteine protease cruzain[35], cruzipain protease[36], dolicholphosphate mannose synthase[37]. On the other hand, many works are devoted to highlighting the antitrypanosomal potency of 1,3,4-thiadiazoles derivatives[38-41]. Despite this, the antitrypanosomal profile of thiadiazole-thiazolidinone dual hybrids is insufficiently covered in the modern scientific literature, which confirms the relevance and novelty of our work. Furthermore, these results prove the necessity of further investigations in order to clarify the features unferlying the antitrypanosomal potential of non-condenced 4-thiazolidinone and 1,3,4-thiadiazole hybrid analogs.

3. Conclusions

A new 2-imino-4-thiazolidinone derivatives incorporated 1,3,4-thiadiazole moiety were synthesized starting from 2-chloro-*N*-(5-ethyl/allylsulfanyl-[1,3,4]thiadiazol-2-yl)-acetamides *via* cyclization under the action of ammonium thiocyanates in acetone medium. Based on Knoevenagel reaction of the synthesized thiadiazolyl-iminothiazolidinones with various carbonyl compounds a series of 5-ylidene derivatives was obtained and some compounds were evaluated for their antitrypanosomal activity against *T. brucei gambience* (Feo strain). Trypanocidal activity assay of the synthesized compounds has allowed us to identify thiazolidinone-thiadiazole hybrids **5a**, **5b**, **6a** and **7a**, which were found to be the most active derivatives, with a range of IC_{50} values = 7.3-12.8 μM. It was established that the presence of 5-ylidene substituent in position 5 of thiazolidinone core and *S*-allyl group in position 5 of thiadiazole cycle is crucial for enhanced antitrypanosomal action of the obtained compounds.

4. Experimental

4.1. Materials and methods

All reagents and solvents were of analytical grade, commercially available and used without further purification and drying. 5-Amino-1,3,4-thiadiazole-2-thiol and 2-amino-5-ethyl-1,3,4-thiadiazole were employed as starting materials and prepared according to known methodologies.[42,43]

Melting points were measured on a NAGEMA-K8 polarization microscope equipped with a Boetius heating stage using a digital thermometer «Ama-digit ad 14 th» and are uncorrected. The ^{1}H NMR spectra were recorded on Varian Gemini 400 MHz in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm units with use of δ scale. The elemental analyses (C, H, N) were performed using on a Elementar Vario L cube instrument. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values.

4.2. Chemistry

*4.2.1. General procedure for the synthesis of 5-unsubstituted 2-(5-allylsulfanyl/ethyl-[1,3,4]thiadiazol-2-ylimino)thiazolidin-4-ones (**4a-b**).*

A mixture of corresponding 2-chloro-*N*-(5-allylsulfanyl/ethyl-[1,3,4]thiadiazol-2-yl)acetamide **1a** or **1b** (0.025 mol) and ammonium thiocyanate (0.05 mol) was heated under the reflux for 8h in dry acetone (80 ml). After cooling to the room temperature the precipitate was filtered off, washed with water and ethanol, dried and recrystallized with acetic acid.

*2-(5-Allylsulfanyl-[1,3,4]thiadiazol-2-ylimino)thiazolidin-4-one (**4a**).* Yield 68%; m.p. = 187–188°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.33 (s, 1H, NH-thiaz), 6.01-5.88 (m, 1H, C<u>H</u>=CH$_2$), 5.32 (d, 1H, *J* = 16.5 Hz, CH=C<u>H</u>$_2$), 5.18 (d, 1H, *J* = 9.9 Hz, CH=C<u>H</u>$_2$), 4.11 (s, 2H, CH$_2$-thiaz), 3.91 (d, 2H, *J* = 6.8 Hz, SCH$_2$). Calcd for $C_8H_8N_4OS_3$: C, 35.28; H, 2.96; N, 20.57. Found: C, 35.43; H, 3.11; N, 20.72.

*2-(5-Ethyl-[1,3,4]thiadiazol-2-ylimino)thiazolidin-4-one (**4b**).* Yield 70%; m.p. = 211–212°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.28 (s, 1H, NH-thiaz), 4.09 (s, 2H, CH$_2$-thiaz), 3.01 (q, 2H, *J* = 7.5 Hz, <u>C</u>H$_2$CH$_3$), 1.30 (t, 3H, *J* = 7.5 Hz, CH$_2$<u>C</u>H$_3$). Calcd for $C_7H_8N_4OS_2$: C, 36.83; H, 3.53; N, 24.54. Found: C, 37.04; H, 3.57; N, 24.72.

*4.2.2. General procedure for the synthesis of 5-arylidene-2-(5-allylsulfanyl/ethyl-[1,3,4]thiadiazol-2-ylimino)thiazolidin-4-ones (**5a-d**).*

A mixture of compounds **4a** or **4b** (3 mmol), appropriate aldehyde (4 mmol) and anhydrous sodium acetate (3 mmol) was refluxed for 4h in glacial acetic acid (20 ml). Powder obtained after cooling was filtered off, washed with acetic acid, water and methanol, dried and recrystallized with acetic acid or DMF:acetic acid (1:2) mixture.

*2-(5-Allylsulfanyl-[1,3,4]thiadiazol-2-ylimino)-5-(4-hydroxybenzylidene)-thiazolidin-4-one (**5a**).* Yield 81%; m.p. = 293–294°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.63 (s, 1H, NH-thiaz), 10.16 (s, 1H, OH), 7.64 (s, 1H, Ar–<u>C</u>H=), 7.46 (d, 2H, *J* = 7.3 Hz, Ar), 6.92 (d, 2H, *J* = 6.9 Hz, Ar), 6.04-5.94 (m, 1H, C<u>H</u>=CH$_2$), 5.34 (d, 1H, *J* = 16.5 Hz, CH=C<u>H</u>$_2$), 5.19 (d, 1H, *J* = 9.8 Hz, CH=C<u>H</u>$_2$), 3.91 (d, 2H, *J* = 5.4 Hz, SCH$_2$). Calcd for $C_{15}H_{12}N_4O_2S_3$: C, 47.86; H, 3.21; N, 14.88. Found: C, 48.05; H, 3.35; N, 14.76.

*2-(5-Allylsulfanyl-[1,3,4]thiadiazol-2-ylimino)-5-(4-methoxybenzylidene)-thiazolidin-4-one (**5b**).* Yield 72%; m.p. = 198–199°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.83 (s, 1H, NH-thiaz), 7.75 (s, 1H, Ar–<u>C</u>H=), 7.63 (d, 2H, *J* = 8.4 Hz, Ar), 7.15 (d, 2H, *J* = 8.4 Hz, Ar), 6.02-5.92 (m, 1H, C<u>H</u>=CH$_2$), 5.34 (d, 1H, *J* = 16.9 Hz, CH=C<u>H</u>$_2$), 5.19 (d, 1H, *J* = 9.9 Hz, CH=C<u>H</u>$_2$), 3.94 (d, 2H, *J* = 6.7 Hz, SCH$_2$), 3.84 (s, 3H, OCH$_3$). Calcd for $C_{16}H_{14}N_4O_2S_3$: C, 49.21; H, 3.61; N, 14.35. Found: C, 49.37; H, 3.74; N, 14.52.

*2-(5-Ethyl-[1,3,4]thiadiazol-2-ylimino)-5-(4-dimethylaminobenzylidene)-thiazolidin-4-one (**5c**).* Yield 74%; m.p. = 271–272°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.60 (s, 1H, NH-thiaz), 7.65 (s, 1H, Ar–CH=), 7.48 (d, 2H, J = 8.6 Hz, Ar), 6.85 (d, 2H, J = 8.5 Hz, Ar), 3.02 (s, 8H, CH_2CH_3, $N(CH_3)_2$), 1.31 (t, 3H, J = 7.5 Hz, CH_2CH_3). Calcd for $C_{16}H_{17}N_5OS_2$: C, 53.46; H, 4.77; N, 19.48. Found: C, 53.65; H, 4.91; N, 19.64.

*2-(5-Ethyl-[1,3,4]thiadiazol-2-ylimino)-5-(4-bromobenzylidene)-thiazolidin-4-one (**5d**).* Yield 70%; m.p. = 312–313°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.92 (s, 1H, NH-thiaz), 7.79 (d, 2H, J = 8.2 Hz, Ar), 7.75 (s, 1H, Ar–CH=), 7.60 (d, 2H, J = 8.2 Hz, Ar), 3.03 (q, 2H, CH_2CH_3), 1.31 (t, 3H, J = 7.5 Hz, CH_2CH_3). Calcd for $C_{14}H_{11}BrN_4OS_2$: C, 42.54; H, 2.80; N, 14.17. Found: C, 42.71; H, 2.93; N, 14.31.

*4.2.3. General procedure for the synthesis of 5-heterylidene- (**6a-c**), 5-isatinylidene- (**7a-b**) or 5-(3-phenyl-allylidene)- (**8a-b**) substituted 2-(5-allylsulfanyl/ethyl-[1,3,4]thiadiazol-2-ylimino)thiazolidin-4-ones*.

A mixture of compounds **4a** or **4b** (3 mmol), appropriate heterylcarbaldehyde, isatin or cinnamaldehyde derivative (4 mmol) and anhydrous sodium acetate (3 mmol) in glacial acetic acid (20 ml) was heated under the reflux for 4h. The reaction mixture was cooled to room temperature, the obtained precipitate was filtered off, washed with acetic acid, water and methanol, dried and recrystallized with acetic acid or DMF:acetic acid (1:2) mixture.

*2-(5-Allylsulfanyl-[1,3,4]thiadiazol-2-ylimino)-5-(thiophene-2-ylmethylene)-thiazolidin-4-one (**6a**).* Yield 77%; m.p. = 297–298°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.92 (s, 1H, NH-thiaz), 8.06-8.03 (m, 2H, thiophene), 7.73 (s, 1H, –CH=), 7.31 (t, 1H, J = 4.4 Hz, thiophene), 6.05-5.90 (m, 1H, $CH=CH_2$), 5.35 (d, 1H, J = 16.3 Hz, $CH=CH_2$), 5.20 (d, 1H, J = 9.8 Hz, $CH=CH_2$), 3.95 (d, 2H, J = 6.8 Hz, SCH_2). Calcd for $C_{13}H_{10}N_4OS_4$: C, 42.60; H, 2.75; N, 15.29. Found: C, 42.79; H, 2.86; N, 15.42.

*2-(5-Ethyl-[1,3,4]thiadiazol-2-ylimino)-5-(thiophene-2-ylmethylene)-thiazolidin-4-one (**6b**).* Yield 80%; m.p. = 264–265°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.82 (s, 1H, NH-thiaz), 8.04-8.03 (m, 2H, thiophene, –CH=), 7.71 (d, 1H, J = 3.0 Hz, thiophene), 7.30 (t, 1H, J = 4.2 Hz, thiophene), 3.04 (q, 2H, J = 7.4 Hz, CH_2CH_3), 1.32 (t, 3H, J = 7.4 Hz, CH_2CH_3). Calcd for $C_{12}H_{10}N_4OS_3$: C, 44.70; H, 3.13; N, 17.38. Found: C, 44.87; H, 3.27; N, 17.54.

*2-(5-Ethyl-[1,3,4]thiadiazol-2-ylimino)-5-(pyridin-3-ylmethylene)-thiazolidin-4-one (**6c**).* Yield 75%; m.p. = 251–252°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.88 (br.s, 1H, NH-thiaz), 8.80 (s, 1H, pyridine), 8.57 (d, 1H, J = 7.5 Hz, pyridine), 7.97 (d, 1H, J = 6.9 Hz, pyridine), 7.74 (s, 1H, –CH=), 7.54 (t, 1H, J = 7.1 Hz, pyridine), 3.02 (q, 2H, J = 7.2 Hz, CH_2CH_3), 1.37 (t, 3H, J = 7.4 Hz, CH_2CH_3). Calcd for $C_{13}H_{11}N_5OS_2$: C, 49.20; H, 3.49; N, 22.07. Found: C, 49.38; H, 3.61; N, 21.94.

*2-(5-Allylsulfanyl-[1,3,4]thiadiazol-2-ylimino)-5-(2-oxo-2,3-dihydroindol-3-ylidene)-thiazolidin-4-one (**7a**).* Yield 84%; m.p. = 294–295°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 13.10 (s, 1H, NH-thiaz), 11.21 (s, 1H, NH-isatin), 8.81 (d, 1H, J = 7.4 Hz, isatin), 7.36 (t, 1H, J = 7.4 Hz, isatin), 7.05 (t, 1H, J = 7.7 Hz, isatin), 6.91 (d, 1H, J = 7.7 Hz, isatin), 6.04-5.90 (m, 1H, $CH=CH_2$), 5.35 (d, 1H, J = 16.6 Hz, $CH=CH_2$), 5.21 (d, 1H, J = 9.8 Hz, $CH=CH_2$), 3.94 (d, 2H, J = 6.6 Hz, SCH_2). Calcd for $C_{16}H_{11}N_5O_2S_3$: C, 47.87; H, 2.76; N, 17.44. Found: C, 48.04; H, 2.89; N, 17.61.

*2-(5-Ethyl-[1,3,4]thiadiazol-2-ylimino)-5-(2-oxo-2,3-dihydroindol-3-ylidene)-thiazolidin-4-one (**7b**).* Yield 87%; m.p. > 350°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 13.05 (s, 1H, NH-thiaz), 11.22 (s, 1H, NH-isatin), 8.82 (d, 1H, J = 7.2 Hz, isatin), 7.38 (t, 1H, J = 7.5 Hz, isatin), 7.07 (t, 1H, J = 7.8 Hz, isatin), 6.93 (d, 1H, J = 7.6 Hz, isatin), 3.04 (q, 2H, J = 7.3 Hz, CH_2CH_3), 1.32 (t, 3H, J = 7.1 Hz, CH_2CH_3). Calcd for $C_{15}H_{11}N_5O_2S_2$: C, 50.41; H, 3.10; N, 19.59. Found: C, 50.58; H, 3.19; N, 19.73.

*2-(5-Ethyl-[1,3,4]thiadiazol-2-ylimino)-5-(3-phenylallylidene)-thiazolidin-4-one (**8a**).* Yield 76%; m.p. 258–259°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.68 (s, 1H, NH-thiaz), 7.72 (d, 2H, J = 7.0 Hz, Ar), 7.47 (d, 1H, J = 11.4 Hz, –CH=), 7.42-7.35 (m, 3H, Ar), 7.30 (d, 1H, J = 15.2 Hz, –CH=), 7.14 (dd, 1H, J_1 = 3.5 Hz, J_2 = 11.6 Hz, –CH=), 3.02 (q, 2H, J = 7.5 Hz, CH_2CH_3), 1.31 (t, 3H, J = 7.5 Hz, CH_2CH_3). Calcd for $C_{16}H_{14}N_4OS_2$: C, 56.12; H, 4.12; N, 16.36. Found: C, 56.26; H, 4.28; N, 16.49.

*2-(5-Ethyl-[1,3,4]thiadiazol-2-ylimino)-5-(2-chloro-3-(4-nitrophenyl)-allylidene)-thiazolidin-4-one (**8b**).* Yield 76%; m.p. 319–320°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 12.86 (br.s, 1H, NH-thiaz), 8.23 (d, 2H, J = 8.4 Hz, Ar), 8.01 (d, 2H, J = 8.3 Hz, Ar), 7.84 (s, 1H, –CH=), 7.65 (s, 1H, –CH=), 3.02 (q, 2H, J = 7.3 Hz, CH_2CH_3), 1.31 (t, 3H, J = 7.4 Hz, CH_2CH_3). Calcd for $C_{16}H_{12}ClN_5O_3S_2$: C, 45.55; H, 2.87; N, 16.60. Found: C, 45.72; H, 2.76; N, 16.76.

4.3. Anti-trypanosomal activity assay

Bloodstream form of *T. brucei gambiense* Feo strain were cultured in HMI9 medium supplemented with 10% FCS at 37ºC under an atmosphere of 5% CO_2.[44] In all experiments, log-phase parasite cultures were harvested by centrifugation at 3000 x 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.[45] Drug stock solutions were prepared in pure DMSO. *T. brucei*

bloodstream forms (10^4 cells) 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 and fluorescence was measured at 530 nm and 590 nm 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. Concentration inhibiting 50% of parasite growth (IC_{50}) was determined from the dose-response curve with a drug concentrations ranging from 10 µg/ml to 0.625 µg/ml and presented in µM. IC_{50} value is the mean ± the standard deviation of three independent experiments.

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Conflicts of Interest

The authors declare no conflict of interest.

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