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Original article

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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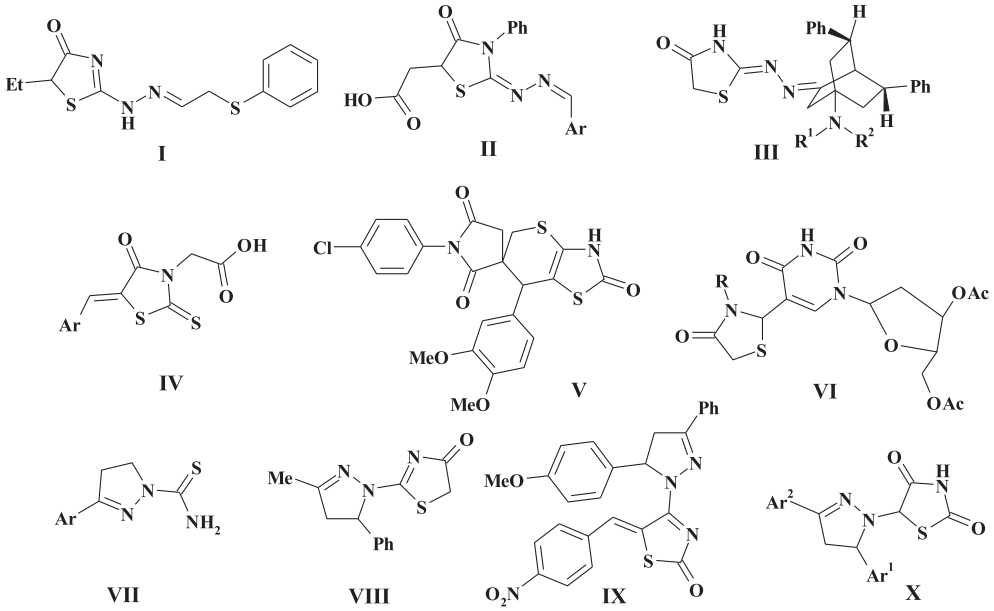


Fig. 1. Thiazolidinones and pyrazolines with trypanocidal activity.

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**.

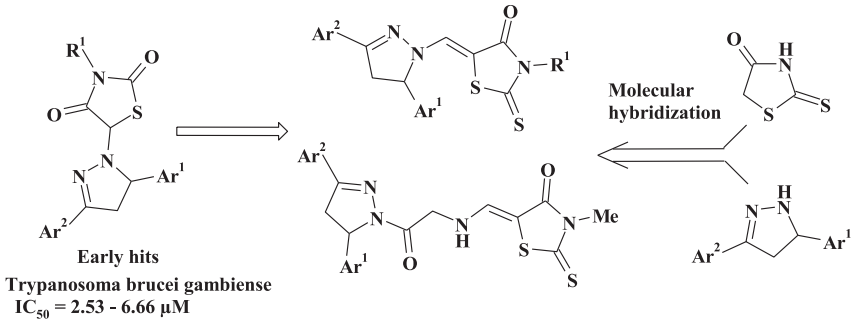
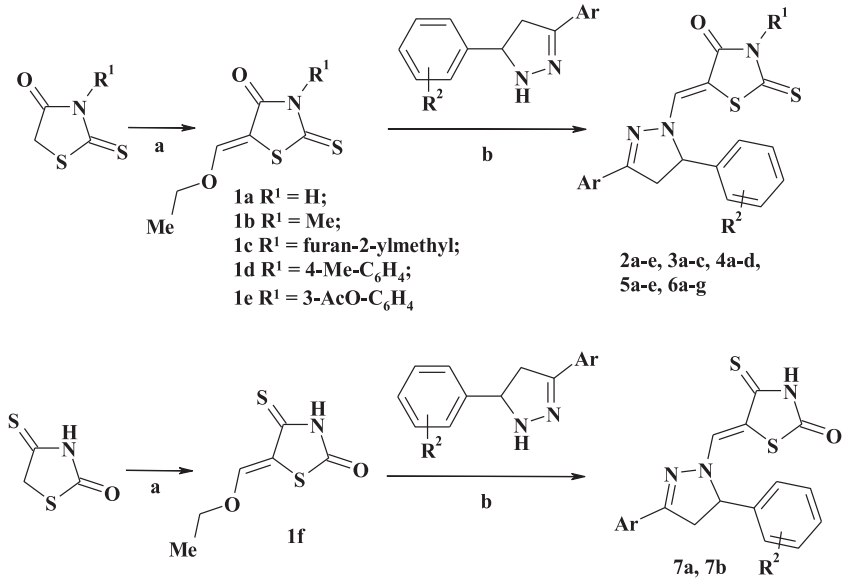


Fig. 2. Design of novel thiazolidinone–pyrazoline hybrids.

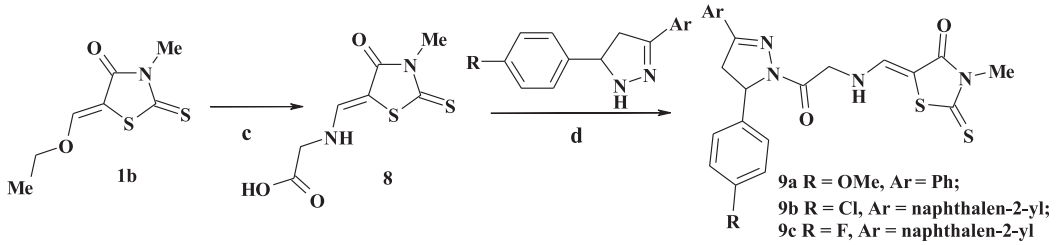
2.2. In vitro evaluation of antitrypanosomal activity and cytotoxicity

All compounds were screened for inhibitory activity against *T. brucei gambiense* (Feo strain). Compounds were first tested at fixed concentrations of 50, 10 and 1 μg/ml. IC_{50} values were further determined for those showing significant trypanocidal activity at 10 μg/ml (>40–50% of parasite growth inhibition) (Table 1). Cytotoxicities against myoblast-derived cell line (L-6) were determined to calculate the selectivity indices (the ratios of cytotoxic CC_{50} values to antitrypanosomal IC_{50} values) of the highly active compounds **3b**, **3c**, **6a**, **6b**, **6d** and **6e**. Nifurtimox, which is used in combination with eflornithine for the gambiense human African trypanosomiasis therapy [29], was selected as reference antiparasitic drug and exhibited a CC_{50} value of 78.2 μM against myoblast-derived cell line (L-6).

Of all the tested compounds, thiazolidinone–pyrazoline hybrids **3c** and **6b** possessed the highest trypanocidal properties, with IC_{50} values of 0.6 μM and 0.7 μM, respectively (Table 1) and 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. The dose–response curves of hybrids **3c**, **6a**, **6b**, **6d** and **6e** are depicted in Fig. 4.



Scheme 1. Synthesis of 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones (**2a**–**e**, **3a**–**c**, **4a**–**d**, **5a**–**e**, **6a**–**g**) and 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-4-thioxothiazolidin-2-ones (**7a**, **7b**). Reagents, conditions and yields: (a) $CH(OC_2H_5)_3$, Ac_2O, reflux 1 h, [27]; (b) EtOH, reflux 1 h, 78–92%.



Scheme 2. Synthesis of 5-{[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxo-ethylamino]-methylene}-3-methyl-2-thioxothiazolidin-4-ones. Reagents, conditions and yields: (c) glycine, AcOH, reflux 3 h, 75%; (d) EtOH, reflux 1 h, 61–75%.

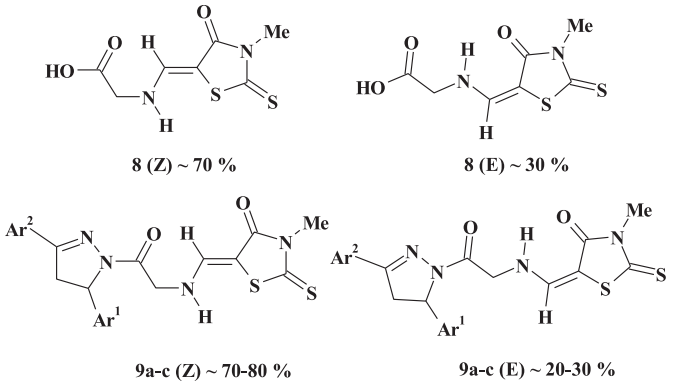


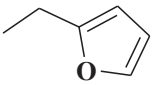
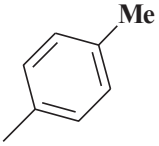
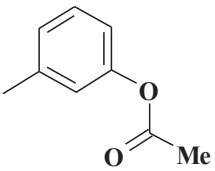
Fig. 3. *Z*/*E*-tautomerism of compounds **8** and **9a**–**c**.

The initial SAR study included two distinct modifications of the thiazolidinone–pyrazoline hybrid structure: variation of substituents at the pyrazoline moiety and substitution of the thiazolidinone NH hydrogen by methyl, 2-furanylmethyl, and aryl groups.

SAR study revealed that aryl variation in the pyrazoline ring had no influence on the antitrypanosomal activity or resulted in ambiguous levels of activity (Table 1). For example, the *N3* unsubstituted thiazolidinones **2a**–**c** with different aryls at 3 and 5 locations of the pyrazoline ring demonstrated the same range of trypanocidal activity (IC_{50} from 10.5 to 14.7 μM). The same trends were observed for the inactive *N3* furan-2-ylmethyl substituted compounds **4a**–**d**. We observed that compounds bearing 2-hydroxyphenyl (**6a**), 4-dimethylaminophenyl (**6e**), and 4-chlorophenyl (**6f**) substituents at position 5 of the 5-aryl-3-phenylpyrazoline fragment were 5–10 fold more potent than the corresponding 4-methoxyphenyl analogue **6c**. Attachment a hydroxyl group to 5-aryl substituent of the 3,5-diarylpyrazoline fragment yielded the most active *N3*-aryl substituted hybrids, as exemplified by **6a** and **6b**. Surprisingly, the replacement of the 4-methoxyphenyl substituent (**5d**) or phenyl substituent (**6f**) at position 3 of the respective 5-(4-chlorophenyl)-3-arylpyrazoline fragments with a 2-naphthalenyl residue (**5e** and **6g**) led to a substantial loss of activity.

Additionally, we observed that the modification of thiazolidinone *N*3 position is more critical for potent trypanocidal activity in comparison to the substitution at positions 3 and 5 derivation of the pyrazoline fragment. Thus, the insertion of a 2-furanylmethyl substituent at the thiazolidinone *N*3 position had an unfavorable effect and led to inactive derivatives (**4a**–**d**). However, the substitution of the thiazolidinone NH hydrogen by methyl or other aryl groups significantly increased trypanocidal potency. *p*-Tolyl substituted hybrid **5a** was 10-fold more potent trypanocidal agent than the parent compound **2a**. A remarkable improvement in activity was observed in the case of the *N*3-(3-acetoxyphenyl) analogues (**6**), indicating that the 3-acetoxyphenyl group was the most beneficial to trypanocidal activity in this series of compounds. Thus, many of the thiazolidinone *N*3 substituted derivatives synthesized

Table 1
Anti-trypanosomal activity and cytotoxicity of thiazolidinone–pyrazoline hybrids.

Comp	R^1	R^2	Ar	% inhibition[a]			IC_{50} μM[b]	SD IC_{50}	Cytotoxicity on myoblast-derived cell line (L-6)		
				50 μg/ml	10 μg/ml	1 μg/ml			CC_{50} μM[b]	SD CC_{50}	SI[d]
2a	H	4-OMe	Ph	91.5	92.3	21.9	14.7	3.79			
2b		4-NMe_2	Ph	74.0	76.2	30.2	10.5	2.2			
2c		4-F	Naphthalen-2-yl	85.4	85.0	19.9	13.2	2.77			
3a[c]	Me	4-Cl	Ph	56.0	42.0	8.2	nd	nd			
3b		4-Cl	Naphthalen-2-yl	97.0	97.2	97.1	>1	–	153.7	4.1	
3c		4-OMe	Naphthalen-2-yl	97.1	97.2	97.2	0.6	0.03	175.2	17.0	292
4a		4-OMe	Ph	46.6	67.3	37.9	nd	nd			
4b		4-OMe	4-OMe-C_6H_4	12.8	48.2	38.7	>98.9	–			
4c		4-OMe	Naphthalen-2-yl	54.6	32.1	19.4	nd	nd			
4d		4-Cl	Naphthalen-2-yl	59.6	45.3	29.0	nd	nd			
5a		4-OMe	Ph	17.8	69.2	52.5	1.5	0.31			
5b		4-OMe	4-OMe-C_6H_4	23.1	55.5	44.5	nd	nd			
5c		4-NMe_2	Ph	5.8	51.9	39.8	>20.1	–			
5d		4-Cl	4-OMe-C_6H_4	40.2	70.4	62.8	2.5	0.81			
5e		4-Cl	Naphthalen-2-yl	41.7	59.4	27.6	21.7	5.74			
6a		2-OH	Ph	90.9	93.8	83.1	1.1	0.18	13.6	0.2	12.4
6b		2-OH	4-OMe-C_6H_4	89.5	91.7	90.1	0.7	0.03	40.0	10.0	57.1
6c		4-OMe	Ph	97.2	97.5	79.3	10.8	0.26			
6d		4-OMe	Naphthalen-2-yl	94.7	95.8	87.6	1.2	0.23	>172.5	–	>143.8
6e		4-NMe_2	Ph	81.6	84.9	53.1	1.2	0.28	>184.3	–	>153.6
6f		4-Cl	Ph	90.3	91.4	42.1	1.9	0.47			
6g		4-Cl	Naphthalen-2-yl	90.6	89.9	66.7	16.5	1.92			
7a	H	2-OH	Ph	85.8	93.3	61.0	3.5	0.46			
7b		4-OMe	Naphthalen-2-yl	87.2	74.7	19.7	18.6	4.71			
9a	Me	4-OMe	Ph	96.1	96.5	62.3	4.6	0.66			
9b		4-Cl	Naphthalen-2-yl	93.5	86.9	52.0	2.1	0.2			
9c		4-F	Naphthalen-2-yl	95.6	94.8	46.6	1.5	0.14			
Nifurtimox							4.4	0.7	78.2	18	17.8

[a] Percentage of inhibition of parasite growth at 50, 10 and 1 μg/ml.
[b] IC_{50} value is the mean ± the standard deviation (SD) of three independent experiments (nd – not determined).
[c] Percentage of inhibition of parasite growth were determined at 100, 20 and 2 μg/ml.
[d] Determined as CC_{50}/IC_{50}.

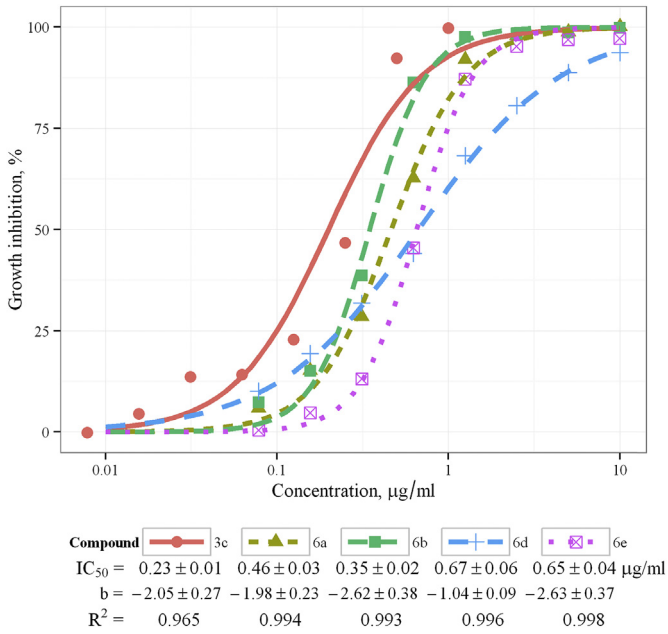


Fig. 4. The dose–response curves of compounds **3c**, **6a**, **6b**, **6d** and **6e** on *Trypanosoma brucei gambiense* growth.

were significantly more active than the *N3* unsubstituted conjugates, and had a range of IC_{50} values of 0.6–1.9 μM (Table 1, Fig. 5).

The further SAR study was directed towards the rhodanine–isorhodanine isomerism. To this end, the isorhodanine–pyrazoline conjugates **7a** and **7b** were synthesized and tested for their trypanocidal activity. The biological activity data revealed that the relative positioning of the thiocarbonyl and carbonyl functions on the thiazolidinone ring (positions 2 and 4) essentially does not affect the activity, considering the same range of the trypanocidal potency of **7b** and isosters **2a**–**c**. However, the 4-thiocarbonyl 4-functionalized derivative **7a** showed a 3–4 fold and 5-fold increase in potency as compared to **2a**–**c** and **7b**, respectively. This finding could be explained by the presence of a hydroxyl group in the diarylpyrazoline moiety, which also provided the benefit of the improved activity in compounds **6a** and **6b** (Table 1, Fig. 5).

Finally, we prepared and tested the 2-oxoethylaminomethylene-linked thiazolidinone–pyrazoline hybrids **9a**–**9c** and observed that elongation of the linker between the two heterocycles decreases the trypanocidal potency as compared to the highly active analogue **3c.**

3. Conclusions

In the present paper new 4-thiazolidinone based conjugates with pyrazoline moiety at 5 position are described. Trypanocidal activity assay of the synthesized compounds has allowed us to identify thiazolidinone–pyrazoline hybrids **3c** and **6b**, which were found to be the most active derivatives, with IC_{50} values of 0.6 μM and 0.7 μM, respectively. A SAR study for the anti-*T. brucei* agents was conducted and included substituent variations at the pyrazoline moiety, modifications of the thiazolidinone *N3* position, elongation of the linker between the two heterocycles, as well as rhodanine–isorhodanine isomerism. We observed a remarkable improvement in activity when the thiazolidinone *N3* position was substituted with methyl and aryl groups. Further investigations on the thiazolidinone–pyrazoline derivatives could lead to more potent compounds as promising candidates for the development of new antitrypanosomal chemotherapy.

4. Experimental

4.1. Materials and methods

2-Thioxo-4-thiazolidinone [30], 4-thioxo-2-thiazolidinone [31], 3,5-diaryl-4,5-dihydro-1*H*-pyrazole [32] and 5-ethoxymethylene rhodanines **1a**–**e** [27] were employed as starting materials and prepared according to the method described previously.

Melting points were measured in open capillary tubes on a BŰCHI B-545 melting point apparatus and are uncorrected. The elemental analysis (C, H, N) were performed using a Perkin–Elmer 2400 CHN analyzer. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. The ^{1}H NMR spectra were recorded on Varian Gemini 400 MHz and ^{13}C NMR spectra on Varian Mercury-400 100 MHz in DMSO-d_6 or DMSO-d_6 + CCl_4 mixture using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm units with use of δ scale. Mass spectra were obtained using electrospray (ES) ionization techniques on an Agilent 1100 Series LCMS. Analytical HPLC were performed on an Agilent 1100 HPLC with DAD (Diode Array Detection). ESI refers to electrospray ionization, HPLC refers to high pressure liquid chromatography, LCMS refers to liquid chromatography coupled with a mass spectrometer, M in the context of mass spectrometry refers to the molecular peak, MS refers to mass spectrometer. Purity of all compounds was determined to be ≥95% by HPLC (Agilent 1100 HPLC) with Diode Array Detection. The peak purity was checked with UV spectra.

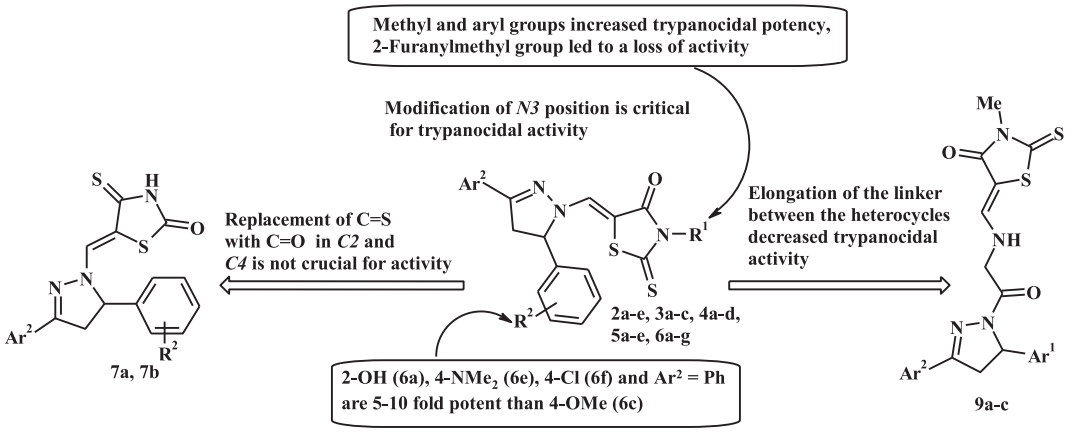


Fig. 5. SAR of trypanocidal thiazolidinone–pyrazoline conjugates.

4.2. Chemistry

4.2.1. General procedure for synthesis of 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones (**2a**–**e**, **3a**–**c**, **4a**–**d**, **5a**–**e**, **6a**–**g**)

A mixture of 5-ethoxymethylene-2-thioxothiazolidin-4-one **1a**–**e** (5 mmol) and appropriate 3,5-diaryl-4,5-dihydro-1*H*-pyrazole (5 mmol) was refluxed in 10 ml of ethanol during 1 h. The crystalline product was separated by filtration, washed with ethanol, and dried. Recrystallization from DMF–EtOH (1:1) rendered the desired product in pure form.

*4.2.1.1. 5-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one (**2a**).* Yield 85%, mp 259–260 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.92 (s, 1H, NH), 7.84–7.89 (m, 2H, arom), 7.56–7.58 (m, 3H, arom), 7.33 (d, 2H, J = 8.5 Hz, arom), 7.24 (s, 1H, =CH), 7.00 (d, 2H, J = 8.5 Hz, arom), 5.58 (dd, 1H, $\underline{CH_2}CH$, J = 11.2, 7.0 Hz), 3.98 (dd, 1H, $\underline{CH_2}CH$, J = 18.2, 11.2 Hz), 3.77 (s, 3H, OCH_3), 3.47 (dd, 1H, $CH_2\underline{CH}$, J = 18.2, 7.0 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 198.2 (C=S), 168.4 (C=O), 159.7 (C=N), 154.7, 144.3, 133.8, 133.2, 130.1, 129.2, 128.6, 127.3, 114.7, 96.5 (=CH), 64.5 ($\underline{C}HCH_2$), 55.3 (OCH_3), 41.4 ($CH\underline{C}H_2$). LCMS (ESI+) m/z 396 $(M+H)^+$. Calcd. for $C_{20}H_{17}N_3O_2S_2$: C, 60.74; H, 4.33; N, 10.62; Found: C, 60.40; H, 4.61; N, 10.38%.

*4.2.1.2. 5-[5-(4-Dimethylaminophenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one (**2b**).* Yield 78%, mp 289–290 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.90 (s, 1H, NH), 7.84–7.87 (m, 2H, arom), 7.56–7.58 (m, 3H, arom), 7.21 (d, 2H, J = 8.8 Hz, arom), 7.19 (s, 1H, =CH), 6.75 (d, 2H, J = 8.8 Hz, arom), 5.48 (dd, 1H, $\underline{CH_2}CH$, J = 11.2, 7.4 Hz), 3.93 (dd, 1H, $\underline{CH_2}CH$, J = 18.3, 11.2 Hz), 3.44 (dd, 1H, $CH_2\underline{CH}$, J = 18.3, 7.4 Hz), 2.91 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 195.9 (C=S), 168.3 (C=O), 159.1 (C=N), 150.7, 132.4, 131.2, 130.3, 129.2, 128.3, 127.2, 126.4, 112.7, 96.2 (=CH), 64.9 ($\underline{C}HCH_2$), 41.2 ($CH\underline{C}H_2$), 39.3 ($N(CH_3)_2$). LCMS (ESI+) m/z 409 $(M+H)^+$. Calcd. for $C_{21}H_{20}N_4OS_2$: C, 61.74; H, 4.93; N, 13.71; Found: C, 61.98; H, 5.15; N, 13.58%.

*4.2.1.3. 5-[5-(4-Fluorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one (**2c**).* Yield 82%, mp 296–298 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.97 (s, 1H, NH), 8.27 (s, 1H, arom), 7.98–8.09 (m, 4H, arom), 7.61–7.63 (m, 2H, arom), 7.46–7.51 (m, 2H, arom), 7.32 (s, 1H, =CH), 7.29 (d, 2H, J = 7.3 Hz, arom), 5.72 (dd, 1H, $\underline{CH_2}CH$, J = 11.5, 6.5 Hz), 4.13 (dd, 1H, $\underline{CH_2}CH$, J = 17.9, 11.5 Hz), 3.60 (dd, 1H, $CH_2\underline{CH}$, J = 17.9, 6.5 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 196.1 (C=S), 168.5 (C=O), 165.8 (d, J_{CF} = 200.0 Hz, 1C), 161.3, 158.8 (C=N), 144.3, 136.5, 134.0, 132.8, 132.5, 131.9 (d, J_{CF} = 30.0 Hz, 2C), 129.5, 129.4, 128.8, 128.7, 128.0, 127.6, 127.2, 123.1, 116.3 (d, J_{CF} = 21.0 Hz, 2C), 97.0 (=CH), 64.3 ($\underline{C}HCH_2$), 41.7 ($CH\underline{C}H_2$). LCMS (ESI+) m/z 434 $(M+H)^+$. Calcd. for $C_{23}H_{16}FN_3OS_2$: C, 63.72; H, 3.72; N, 9.69; Found: C, 63.50; H, 3.45; N, 9.98%.

*4.2.1.4. 5-[5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-methyl-2-thioxothiazolidin-4-one (**3a**).* Yield 87%, mp 288–290 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.84–7.86 (m, 2H, arom), 7.50–7.58 (m, 6H, arom, =CH), 7.40–7.42 (m, 2H, arom), 5.72 (dd, 1H, $\underline{CH_2}CH$, J = 11.3, 6.0 Hz), 4.04 (dd, 1H, $\underline{CH_2}CH$, J = 18.1, 11.3 Hz), 3.48 (dd, 1H, $CH_2\underline{CH}$, J = 18.1, 6.0 Hz), 3.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.8 (C=S), 166.3 (C=O), 159.4 (C=N), 144.3, 139.3, 133.7, 133.5, 131.4, 129.9, 129.4, 129.2, 129.0, 127.4, 94.0 (=CH), 64.2 ($\underline{C}HCH_2$), 41.7 ($CH\underline{C}H_2$), 30.8 (CH_3). LCMS (ESI+) m/z 414/416 $(M+H)^+$. Calcd. for $C_{20}H_{16}ClN_3OS_2$: C, 58.03; H, 3.90; N, 8.56; Found: C, 58.37; H, 3.67; N, 8.82%.

*4.2.1.5. 5-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-3-methyl-2-thioxothiazolidin-4-one (**3b**).* Yield 80%, mp 279–280 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.26 (s, 1H, arom), 8.08 (brs, 2H, arom), 7.97–8.02 (m, 2H, arom), 7.61–7.63 (m, 2H, arom), 7.57 (s, 1H, =CH), 7.52 (d, 2H, J = 8.4 Hz, arom), 7.44 (d, 2H, J = 8.4 Hz, arom), 5.76 (dd, 1H, $\underline{CH_2}CH$, J = 11.4, 6.1 Hz), 4.14 (dd, 1H, $\underline{CH_2}CH$, J = 18.4, 11.4 Hz), 3.61 (dd, 1H, $CH_2\underline{CH}$, J = 18.4, 6.1 Hz), 3.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.5 (C=S), 166.3 (C=O), 159.4 (C=N), 144.3, 139.3, 134.1, 133.6, 133.5, 132.7, 129.4, 129.0, 128.9, 128.8, 128.7, 128.0, 127.9, 127.5, 127.2, 123.1, 94.2 (=CH), 64.3 ($\underline{C}HCH_2$), 41.7 ($CH\underline{C}H_2$), 30.8 (CH_3). LCMS (ESI+) m/z 464/465 $(M+H)^+$. Calcd. for $C_{24}H_{18}ClN_3OS_2$: C, 62.13; H, 3.91; N, 9.06; Found: C, 62.37; H, 4.18; N, 9.32%.

*4.2.1.6. 5-[5-(4-Methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-3-methyl-2-thioxothiazolidin-4-one (**3c**).* Yield 86%, mp 250–252 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.28 (s, 1H, arom), 8.08 (brs, 2H, arom), 7.97–8.03 (m, 2H, arom), 7.61–7.63 (m, 2H, arom), 7.45 (s, 1H, =CH), 7.37 (d, 2H, J = 8.4 Hz, arom), 7.00 (d, 2H, J = 8.4 Hz, arom), 5.66 (dd, 1H, $\underline{CH_2}CH$, J = 11.2, 6.7 Hz), 4.10 (dd, 1H, $\underline{CH_2}CH$, J = 18.1, 11.2 Hz), 3.77 (s, 3H, OCH_3), 3.60 (dd, 1H, $CH_2\underline{CH}$, J = 18.2, 6.7 Hz), 3.28 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.6 (C=S), 186.4 (C=O), 166.2, 159.6 (C=N), 134.1, 133.4, 132.8, 131.9, 128.9, 128.8, 128.7, 128.6, 128.0, 127.6, 127.2, 123.1, 114.8, 93.8 (=CH), 64.7 ($\underline{C}HCH_2$), 55.3 (OCH_3), 41.5 ($CH\underline{C}H_2$), 30.8 (CH_3). LCMS (ESI+) m/z 460 $(M+H)^+$. Calcd. for $C_{25}H_{21}N_3O_2S_2$: C, 65.34; H, 4.61; N, 9.14; Found: C, 65.67; H, 4.39; N, 9.37%.

*4.2.1.7. 3-Furan-2-ylmethyl-5-[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one (**4a**).* Yield 75%, mp 198–200 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.87–7.89 (m, 2H, arom), 7.54–7.59 (m, 4H, arom), 7.43 (s, 1H, =CH), 7.35 (d, 2H, J = 8.1 Hz, arom), 7.01 (d, 2H, J = 8.1 Hz, arom), 6.38 (brs, 1H, arom), 6.31 (brs, 1H, arom), 5.61 (dd, 1H, $\underline{CH_2}CH$, J = 11.3, 6.5 Hz), 5.13 (s, 2H, CH_2), 4.01 (dd, 1H, $\underline{CH_2}CH$, J = 18.2, 11.3 Hz), 3.78 (s, 3H, OCH_3), 3.50 (dd, 1H, $CH_2\underline{CH}$, J = 18.2, 6.5 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.2 (C=S), 165.6 (C=O), 159.9, 159.7 (C=N), 148.6, 142.5, 133.7, 131.7, 131.4, 130.0, 129.2, 128.7, 127.4, 114.8, 110.6, 108.9, 92.9 (=CH), 64.6 ($\underline{C}HCH_2$), 55.3 (OCH_3), 41.5 ($CH\underline{C}H_2$), 40.5 (CH_2). LCMS (ESI+) m/z 476 $(M+H)^+$. Calcd. for $C_{25}H_{21}N_3O_3S_2$: C, 63.14; H, 4.45; N, 8.84; Found: C, 62.82; H, 4.12; N, 8.55%.

*4.2.1.8. 5-[3,5-Bis-(4-methoxyphenyl)-4,5-dihydropyrazol-1-ylmethylene]-3-furan-2-ylmethyl-2-thioxothiazolidin-4-one (**4b**).* Yield 88%, mp 262–264 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.82 (d, 2H, J = 8.6 Hz, arom), 7.53 (s, 1H, arom), 7.40 (s, 1H, =CH), 7.33 (d, 2H, J = 8.5 Hz, arom), 7.14 (d, 2H, J = 8.6 Hz, arom), 6.99 (d, 2H, J = 8.5 Hz, arom), 6.36 (brs, 1H, arom), 6.28 (d, 1H, J = 2.8 Hz, arom), 5.59 (dd, 1H, $\underline{CH_2}CH$, J = 11.1, 7.1 Hz), 5.12 (s, 2H, CH_2), 3.96 (dd, 1H, $\underline{CH_2}CH$, J = 18.3, 11.1 Hz), 3.84 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.46 (dd, 1H, $CH_2\underline{CH}$, J = 18.3, 7.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 192.9 (C=S), 165.5 (C=O), 161.9, 159.9, 159.7 (C=N), 148.7, 142.5, 133.6, 131.7, 129.3, 128.6, 122.4, 114.8, 110.6, 108.9, 92.2 (=CH), 64.5 ($\underline{C}HCH_2$), 55.6 (OCH_3), 55.3 (OCH_3), 41.6 ($CH\underline{C}H_2$), 40.5 (CH_2). LCMS (ESI+) m/z 506 $(M+H)^+$. Calcd. for $C_{26}H_{23}N_3O_4S_2$: C, 61.76; H, 4.59; N, 8.31; Found: C, 61.48; H, 4.75; N, 8.09%.

*4.2.1.9. 3-Furan-2-ylmethyl-5-[5-(4-methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one (**4c**).* Yield 91%, mp 224–226 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.28 (s, 1H, arom), 8.08 (brs, 2H, arom), 7.97–8.04 (m, 2H, arom), 7.60–7.63 (m, 2H, arom), 7.55 (brs, 1H, arom), 7.45 (s, 1H, =CH), 7.37 (d, 2H, J = 8.6 Hz, arom), 7.01 (d, 2H, J = 8.6 Hz, arom),

6.36 (t, 1H, $J = 3.0$ Hz, arom), 6.31 (d, 1H, $J = 3.0$ Hz, arom), 5.65 (dd, 1H, CH_2CH, $J = 11.1$, 6.7 Hz), 5.13 (s, 2H, CH_2), 4.10 (dd, 1H, CH_2CH, $J = 18.2$, 11.1 Hz), 3.77 (s, 3H, OCH_3), 3.61 (dd, 1H, CH_2CH, $J = 18.2$, 6.7 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.2 (C=S), 165.7 (C=O), 159.9, 159.7 (C=N), 148.7, 142.5, 134.1, 133.6, 132.8, 131.7, 129.0, 128.8, 128.7, 128.6, 128.0, 127.9, 127.6, 127.2, 123.1, 114.8, 110.6, 108.9, 93.1 (=CH), 64.7 ($CHCH_2$), 55.3 (OCH_3), 41.5 ($CHCH_2$), 40.6 (CH_2). LCMS (ESI+) m/z 526 $(M+H)^+$. Calcd. for $C_{29}H_{23}N_3O_3S_2$: C, 66.26; H, 4.41; N, 7.99; Found: C, 66.01; H, 4.68; N, 8.27%.

*4.2.1.10. 3-Furan-2-ylmethyl-5-[5-(4-chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxothiazolidin-4-one (**4d**).* Yield 82%, mp 232–234 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.28 (s, 1H, arom), 8.09 (brs, 2H, arom), 7.97–8.03 (m, 2H, arom), 7.60–7.65 (m, 2H, arom), 7.51–7.55 (m, 4H, arom, =CH), 7.46 (d, 2H, $J = 7.1$ Hz, arom), 6.38 (brs, 1H, arom), 6.31 (brs, 1H, arom), 5.78 (dd, 1H, CH_2CH, $J = 10.9$, 6.3 Hz), 5.20 (s, 2H, CH_2), 4.17 (dd, 1H, CH_2CH, $J = 18.0$, 10.9 Hz), 3.62 (dd, 1H, CH_2CH, $J = 18.0$, 6.3 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.3 (C=S), 165.7 (C=O), 159.7 (C=N), 148.7, 144.3, 142.6, 139.2, 134.1, 133.9, 133.5, 132.7, 129.4, 129.0, 128.9, 128.8, 128.0, 127.9, 127.4, 127.2, 123.1, 110.7, 109.0, 93.6 (=CH), 64.4 ($CHCH_2$), 41.7 ($CHCH_2$), 40.5 (CH_2). LCMS (ESI+) m/z 531 $(M+H)^+$. Calcd. for $C_{28}H_{20}ClN_3O_2S_2$: C, 63.45; H, 3.80; N, 7.93; Found: C, 63.72; H, 4.12; N, 7.68%.

*4.2.1.11. 5-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxo-3-p-tolylthiazolidin-4-one (**5a**).* Yield 88%, mp 214–216 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.89–7.91 (m, 2H, arom), 7.58–7.61 (m, 3H, arom), 7.43 (s, 1H, =CH), 7.35 (d, 2H, $J = 8.6$ Hz, arom), 7.29 (d, 2H, $J = 8.1$ Hz, arom), 7.11 (d, 2H, $J = 8.1$ Hz, arom), 7.00 (d, 2H, $J = 8.6$ Hz, arom), 5.63 (dd, 1H, CH_2CH, $J = 11.5$, 6.7 Hz), 4.03 (dd, 1H, CH_2CH, $J = 18.4$, 11.5 Hz), 3.76 (s, 3H, OCH_3), 3.50 (dd, 1H, CH_2CH, $J = 18.4$, 6.7 Hz), 2.35 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.4 (C=S), 166.3 (C=O), 159.7 (C=N), 159.6, 144.3, 138.5, 133.6, 133.4, 131.9, 131.4, 130.1, 129.7, 129.2, 128.6, 128.5, 127.4, 114.8, 93.9 (=CH), 64.6 ($CHCH_2$), 55.3 (OCH_3), 41.5 ($CHCH_2$), 20.9 (CH_3). LCMS (ESI+) m/z 486 $(M+H)^+$. Calcd. for $C_{27}H_{23}N_3O_2S_2$: C, 66.78; H, 4.77; N, 8.65; Found: C, 66.43; H, 4.99; N, 8.42%.

*4.2.1.12. 5-[3,5-Bis-(4-methoxyphenyl)-4,5-dihydropyrazol-1-ylmethylene]-2-thioxo-3-p-tolylthiazolidin-4-one (**5b**).* Yield 92%, mp 270–272 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.84 (d, 2H, $J = 8.7$ Hz, arom), 7.41 (s, 1H, =CH), 7.33 (d, 2H, $J = 8.6$ Hz, arom), 7.28 (d, 2H, $J = 8.2$ Hz, arom), 7.15 (d, 2H, $J = 8.7$ Hz, arom), 7.10 (d, 2H, $J = 8.2$ Hz, arom), 6.99 (d, 2H, $J = 8.6$ Hz, arom), 5.59 (dd, 1H, CH_2CH, $J = 11.2$, 6.7 Hz), 3.97 (dd, 1H, CH_2CH, $J = 18.2$, 11.2 Hz), 3.84 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.46 (dd, 1H, CH_2CH, $J = 18.2$, 6.7 Hz), 2.35 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.2 (C=S), 166.2 (C=O), 161.9, 159.7 (C=N), 159.6, 138.4, 133.7, 133.4, 131.9, 129.6, 129.3, 128.6, 128.5, 122.5, 114.8, 93.2 (=CH), 64.5 ($CHCH_2$), 55.6 (OCH_3), 55.3 (OCH_3), 41.5 ($CHCH_2$), 20.9 (CH_3). LCMS (ESI+) m/z 516 $(M+H)^+$. Calcd. for $C_{28}H_{25}N_3O_3S_2$: C, 65.22; H, 4.89; N, 8.15; Found: C, 65.03; H, 4.63; N, 8.39%.

*4.2.1.13. 5-[5-(4-Dimethylaminophenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxo-3-p-tolylthiazolidin-4-one (**5c**).* Yield 81%, mp 164–166 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.90 (d, 2H, $J = 6.5$ Hz, arom), 7.57–7.62 (m, 3H, arom), 7.38 (s, 1H, =CH), 7.28 (d, 2H, $J = 7.6$ Hz, arom), 7.22 (d, 2H, $J = 7.8$ Hz, arom), 7.11 (d, 2H, $J = 7.4$ Hz, arom), 6.75 (d, 2H, $J = 7.8$ Hz, arom), 5.54 (dd, 1H, CH_2CH, $J = 10.6$, 6.8 Hz), 3.98 (dd, 1H, CH_2CH, $J = 18.6$, 10.6 Hz), 3.49 (dd, 1H, CH_2CH, $J = 18.6$, 6.8 Hz), 2.90 (s, 6H, $N(CH_3)_2$), 2.35 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.4 (C=S), 166.2 (C=O), 159.7 (C=N), 150.7, 138.5, 133.6, 133.3, 131.3, 130.2, 129.6, 129.2, 128.5, 128.1, 127.3, 126.5, 112.7, 93.6 (=CH), 65.0 ($CHCH_2$), 41.3 ($CHCH_2$), 39.3 ($N(CH_3)_2$), 20.9 (CH_3). LCMS (ESI+) m/z 499 $(M+H)^+$. Calcd. for $C_{28}H_{26}N_4OS_2$: C, 67.44; H, 5.26; N, 11.24; Found: C, 67.70; H, 5.48; N, 11.52%.

*4.2.1.14. 5-[5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-ylmethylene]-2-thioxo-3-p-tolylthiazolidin-4-one (**5d**).* Yield 85%, mp 210–211 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.88 (d, 2H, $J = 8.5$ Hz, arom), 7.66 (d, 2H, $J = 8.5$ Hz, arom), 7.41 (s, 1H, =CH), 7.35 (d, 2H, $J = 8.6$ Hz, arom), 7.28 (d, 2H, $J = 8.1$ Hz, arom), 7.11 (d, 2H, $J = 8.1$ Hz, arom), 7.00 (d, 2H, $J = 8.6$ Hz, arom), 5.63 (dd, 1H, CH_2CH, $J = 11.3$, 7.0 Hz), 3.99 (dd, 1H, CH_2CH, $J = 18.4$, 11.3 Hz), 3.76 (s, 3H, OCH_3), 3.49 (dd, 1H, CH_2CH, $J = 18.4$, 7.0 Hz), 2.35 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.4 (C=S), 166.3 (C=O), 159.7 (C=N), 158.5, 138.5, 135.9, 133.6, 133.3, 131.8, 129.7, 129.4, 129.0, 128.7, 128.5, 114.7, 94.3 (=CH), 64.8 ($CHCH_2$), 55.3 (OCH_3), 41.4 ($CHCH_2$), 20.9 (CH_3). LCMS (ESI+) m/z 520/522 $(M+H)^+$. Calcd. for $C_{27}H_{22}ClN_3O_2S_2$: C, 62.36; H, 4.26; N, 8.08; Found: C, 62.61; H, 4.01; N, 8.27%.

*4.2.1.15. 5-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-2-thioxo-3-p-tolylthiazolidin-4-one (**5e**).* Yield 78%, mp 262–264 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.29 (s, 1H, arom), 8.12 (brs, 2H, arom), 7.98–8.04 (m, 2H, arom), 7.62–7.65 (m, 2H, arom), 7.57 (s, 1H, =CH), 7.52 (d, 2H, $J = 8.4$ Hz, arom), 7.45 (d, 2H, $J = 8.4$ Hz, arom), 7.30 (d, 2H, $J = 8.1$ Hz, arom), 7.13 (d, 2H, $J = 8.1$ Hz, arom), 5.78 (dd, 1H, CH_2CH, $J = 11.4$, 6.0 Hz), 4.17 (dd, 1H, CH_2CH, $J = 18.4$, 11.4 Hz), 3.62 (dd, 1H, CH_2CH, $J = 18.4$, 6.0 Hz), 2.36 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.5 (C=S), 166.4 (C=O), 159.4 (C=N), 139.4, 138.5, 134.1, 133.6, 133.5, 133.4, 132.8, 129.7, 129.4, 129.0, 128.9, 128.7, 128.5, 128.0, 127.9, 127.5, 127.3, 123.1, 94.5 (=CH), 64.3 ($CHCH_2$), 40.5 ($CHCH_2$), 20.9 (CH_3). LCMS (ESI+) m/z 541/543 $(M+H)^+$. Calcd. for $C_{30}H_{22}ClN_3OS_2$: C, 66.71; H, 4.11; N, 7.78; Found: C, 66.48; H, 3.87; N, 7.52%.

*4.2.1.16. 5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**6a**).* Yield 78%, mp 238–240 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 9.94 (s, 1H, OH), 7.89 (d, 2H, $J = 5.8$ Hz, arom), 7.51–7.55 (m, 3H, arom), 7.46 (s, 1H, =CH), 7.19 (d, 2H, $J = 7.4$ Hz, arom), 7.12 (d, 1H, $J = 7.6$ Hz, arom), 7.02 (s, 1H, arom), 6.90 (d, 1H, $J = 7.9$ Hz, arom), 6.82 (t, 1H, $J = 7.1$ Hz, arom), 5.75 (dd, 1H, CH_2CH, $J = 11.5$, 5.9 Hz), 3.88 (dd, 1H, CH_2CH, $J = 18.1$, 11.5 Hz), 3.51 (dd, 1H, CH_2CH, $J = 18.1$, 5.9 Hz), 2.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.0 (C=S), 169.0 (C=O), 166.0 (C=O), 160.1, 155.4 (C=N), 150.8, 144.3, 137.0, 133.4, 131.3, 130.2, 129.8, 129.2, 127.3, 126.4, 125.1, 122.6, 122.5, 119.5, 116.1, 93.3 (=CH), 62.1 ($CHCH_2$), 40.5 ($CHCH_2$), 20.9 (CH_3). LCMS (ESI+) m/z 516 $(M+H)^+$. Calcd. for $C_{27}H_{21}N_3O_4S_2$: C, 62.90; H, 4.11; N, 8.15; Found: C, 62.63; H, 4.32; N, 8.40%.

*4.2.1.17. 5-[5-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**6b**).* Yield 82%, mp 266–268 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 10.28 (s, 1H, OH), 7.81 (d, 2H, $J = 8.4$ Hz, arom), 7.48–7.53 (m, 2H, arom), 7.36 (s, 1H, =CH), 7.29 (d, 1H, $J = 8.4$ Hz, arom), 7.19 (d, 1H, $J = 7.8$ Hz, arom), 7.11 (d, 1H, $J = 7.8$ Hz, arom), 7.06 (d, 2H, $J = 8.4$ Hz, arom), 7.03 (s, 1H, arom), 6.84 (d, 1H, $J = 8.5$ Hz, arom), 5.68 (dd, 1H, CH_2CH, $J = 11.4$, 6.4 Hz), 3.86 (s, 3H, OCH_3), 3.83 (dd, 1H, CH_2CH, $J = 17.9$, 11.4 Hz), 3.47 (dd, 1H, CH_2CH, $J = 17.9$, 6.4 Hz), 2.28 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.8 (C=S), 169.0 (C=O), 166.0 (C=O), 161.8, 160.0, 154.9 (C=N), 150.7, 137.0, 133.3, 132.7, 131.7, 129.7, 129.2, 127.6, 126.4, 122.6, 122.5, 118.3, 114.7, 110.2, 92.8 (=CH), 61.5 ($CHCH_2$), 55.6 (OCH_3), 40.5 ($CHCH_2$), 20.9 (CH_3). LCMS (ESI+) m/z 546

$(M+H)^+$. Calcd. for $C_{28}H_{23}N_3O_5S_2$: C, 61.64; H, 4.25; N, 7.70; Found: C, 61.38; H, 4.05; N, 7.93%.

*4.2.1.18. 5-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**6c**).* Yield 77%, mp 218–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.89–7.92 (m, 2H, arom), 7.56–7.60 (m, 3H, arom), 7.52 (d, 1H, J = 8.0 Hz, arom), 7.45 (s, 1H, =CH), 7.35 (d, 2H, J = 8.6 Hz, arom), 7.24 (d, 1H, J = 8.2 Hz, arom), 7.18 (d, 1H, J = 8.0 Hz, arom), 7.12 (brs, 1H, arom), 7.00 (d, 2H, J = 8.6 Hz, arom), 5.64 (dd, 1H, $\underline{CH_2}CH$, J = 11.4, 6.7 Hz), 4.03 (dd, 1H, $\underline{CH_2}CH$, J = 18.5, 11.4 Hz), 3.76 (s, 3H, OCH_3), 3.51 (dd, 1H, $CH_2\underline{CH}$, J = 18.5, 6.7 Hz), 2.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.1 (C=S), 169.0 (C=O), 166.0 (C=O), 159.8, 159.7, 150.8 (C=N), 144.3, 136.9, 133.5, 131.9, 131.4, 130.1, 129.8, 129.2, 128.6, 127.4, 122.5, 114.8, 93.8 (=CH), 64.6 ($\underline{C}HCH_2$), 55.3 (OCH_3), 41.5 ($CH\underline{C}H_2$), 20.9 (CH_3). LCMS (ESI+) m/z 530 $(M+H)^+$. Calcd. for $C_{28}H_{23}N_3O_4S_2$: C, 63.50; H, 4.38; N, 7.93; Found: C, 63.22; H, 4.65; N, 7.71%.

*4.2.1.19. 5-[5-(4-Methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**6d**).* Yield 89%, mp 232–234 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.31 (s, 1H, arom), 8.12 (brs, 2H, arom), 8.00 (t, 2H, J = 7.9 Hz, arom), 7.62–7.65 (m, 2H, arom), 7.49–7.55 (m, 2H, arom, =CH), 7.38 (d, 2H, J = 8.4 Hz, arom), 7.25 (d, 1H, J = 7.8 Hz, arom), 7.19 (d, 1H, J = 7.8 Hz, arom), 7.14 (brs, 1H, arom), 7.01 (d, 2H, J = 8.4 Hz, arom), 5.68 (dd, 1H, $\underline{CH_2}CH$, J = 11.1, 6.8 Hz), 4.13 (dd, 1H, $\underline{CH_2}CH$, J = 18.1, 11.1 Hz), 3.77 (s, 3H, OCH_3), 3.63 (dd, 1H, $CH_2\underline{CH}$, J = 18.1, 6.8 Hz), 2.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.1 (C=S), 169.0 (C=O), 166.0 (C=O), 159.8, 159.7, 150.8 (C=N), 136.9, 134.1, 133.5, 132.8, 131.9, 129.8, 129.0, 128.9, 128.8, 128.6, 128.0, 127.9, 127.6, 127.2, 126.4, 123.1, 122.6, 122.5, 114.8, 94.0 (=CH), 64.8 ($\underline{C}HCH_2$), 55.3 (OCH_3), 41.5 ($CH\underline{C}H_2$), 20.9 (CH_3). LCMS (ESI+) m/z 580 $(M+H)^+$. Calcd. for $C_{32}H_{25}N_3O_4S_2$: C, 66.30; H, 4.35; N, 7.25; Found: C, 66.57; H, 4.59; N, 7.03%.

*4.2.1.20. 5-[5-(4-Dimethylaminophenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**6e**).* Yield 84%, mp 252–254 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.86–7.89 (m, 2H, arom), 7.48–7.55 (m, 4H, arom), 7.39 (s, 1H, =CH), 7.19 (d, 2H, J = 8.2 Hz, arom), 7.10 (d, 1H, J = 7.4 Hz, arom), 7.02 (brs, 1H, arom), 6.73 (d, 2H, J = 8.2 Hz, arom), 5.52 (dd, 1H, $\underline{CH_2}CH$, J = 11.5, 6.4 Hz), 3.95 (dd, 1H, $\underline{CH_2}CH$, J = 18.0, 11.5 Hz), 3.41 (dd, 1H, $CH_2\underline{CH}$, J = 18.0, 6.4 Hz), 2.95 (s, 6H, $N(CH_3)_2$), 2.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.0 (C=S), 169.0 (C=O), 166.0 (C=O), 159.9, 150.8 (C=N), 150.7, 136.9, 133.5, 131.4, 130.2, 129.8, 129.2, 128.1, 127.4, 126.5, 126.4, 122.6, 122.5, 112.7, 93.6 (=CH), 65.0 ($\underline{C}HCH_2$), 41.3 ($CH\underline{C}H_2$), 39.4 ($N(CH_3)_2$), 20.9 (CH_3). LCMS (ESI+) m/z 543 $(M+H)^+$. Calcd. for $C_{29}H_{26}N_4O_3S_2$: C, 64.19; H, 4.83; N, 10.32; Found: C, 64.35; H, 4.61; N, 10.62%.

*4.2.1.21. 5-[5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**6f**).* Yield 80%, mp 264–266 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.87–7.90 (m, 2H, arom), 7.51–7.60 (m, 7H, arom, =CH), 7.42 (d, 2H, J = 8.1 Hz, arom), 7.25 (d, 1H, J = 7.2 Hz, arom), 7.18 (d, 1H, J = 7.2 Hz, arom), 7.13 (brs, 1H, arom), 5.74 (dd, 1H, $\underline{CH_2}CH$, J = 11.4, 5.8 Hz), 4.07 (dd, 1H, $\underline{CH_2}CH$, J = 18.6, 11.4 Hz), 3.51 (dd, 1H, $CH_2\underline{CH}$, J = 18.6, 5.8 Hz), 2.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.3 (C=S), 169.0 (C=O), 166.0 (C=O), 159.5, 150.8 (C=N), 144.3, 139.3, 136.9, 133.8, 133.5, 131.5, 130.0, 129.8, 129.4, 129.3, 129.0, 127.4, 126.4, 122.7, 122.6, 94.3 (=CH), 64.2 ($\underline{C}HCH_2$), 41.7 ($CH\underline{C}H_2$), 20.9 (CH_3). LCMS (ESI+) m/z 534/536 $(M+H)^+$. Calcd. for $C_{27}H_{20}ClN_3O_3S_2$: C, 60.72; H, 3.77; N, 7.87; Found: C, 60.43; H, 3.95; N, 7.55%.

*4.2.1.22. 5-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-3-(3-acetoxyphenyl)-2-thioxothiazolidin-4-one (**6g**).* Yield 91%, mp 260–262 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.30 (s, 1H, arom), 8.12 (brs, 2H, arom), 7.98–8.04 (m, 2H, arom), 7.57–7.64 (m, 4H, arom, =CH), 7.53 (d, 2H, J = 8.9 Hz, arom), 7.45 (d, 2H, J = 8.4 Hz, arom), 7.26 (d, 1H, J = 7.1 Hz, arom), 7.20 (d, 1H, J = 8.2 Hz, arom), 7.14 (brs, 1H, arom), 5.79 (dd, 1H, $\underline{CH_2}CH$, J = 11.3, 6.8 Hz), 4.17 (dd, 1H, $\underline{CH_2}CH$, J = 18.1, 11.3 Hz), 3.63 (dd, 1H, $CH_2\underline{CH}$, J = 18.1, 5.6 Hz), 2.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 194.2 (C=S), 169.0 (C=O), 166.0 (C=O), 159.5, 150.9 (C=N), 139.4, 136.9, 134.1, 133.7, 133.5, 132.8, 129.8, 129.4, 129.0, 128.9, 128.8, 128.7, 128.0, 127.9, 127.5, 127.2, 126.4, 123.1, 122.7, 122.6, 94.5 (=CH), 64.4 ($\underline{C}HCH_2$), 41.7 ($CH\underline{C}H_2$), 20.9 (CH_3). LCMS (ESI+) m/z 584/586 $(M+H)^+$. Calcd. for $C_{31}H_{22}ClN_3O_3S_2$: C, 63.74; H, 3.80; N, 7.19; Found: C, 63.92; H, 3.61; N, 7.45%.

*4.2.2. General procedure for synthesis of 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-4-thioxothiazolidin-2-ones (**7a**, **7b**)*

A mixture of 5-ethoxymethylene-4-thioxothiazolidin-2-one **1f** (5 mmol) and appropriate 3,5-diaryl-4,5-dihydropyrazole (5 mmol) was refluxed in 10 ml of ethanol during 1 h. After cooling, the solid precipitated was filtered off, washed with ethanol and recrystallized from a DMF/ethanol 1:2 mixture.

*4.2.2.1. 5-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-4-thioxothiazolidin-2-one (**7a**).* Yield 91%, mp 260–261 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.32 (s, 1H, NH), 9.93 (s, 1H, OH), 7.98 (d, 1H, J = 9.8 Hz, arom), 7.88 (brs, 2H, arom), 7.48–7.57 (m, 3H, arom, =CH), 7.24–7.28 (m, 2H, arom), 6.88–6.94 (m, 2H, arom), 5.84 (dd, 1H, $\underline{CH_2}CH$, J = 11.8, 6.1 Hz), 3.90 (dd, 1H, $\underline{CH_2}CH$, J = 18.0, 11.8 Hz), 3.54 (dd, 1H, $CH_2\underline{CH}$, J = 18.0, 6.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.3 (C=S), 172.5 (C=O), 160.8 (C=N), 155.5, 137.0, 131.5, 130.4, 130.1, 129.4, 129.2, 127.5, 124.7, 119.5, 116.2, 104.4 (=CH), 62.8 ($\underline{C}HCH_2$), 41.3 ($CH\underline{C}H_2$). LCMS (ESI+) m/z 382 $(M+H)^+$. Calcd. for $C_{19}H_{15}N_3O_2S_2$: C, 59.82; H, 3.96; N, 11.02; Found: C, 60.07; H, 3.71; N, 10.79%.

*4.2.2.2. 5-[5-(4-Methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-4-thioxothiazolidin-2-one (**7b**).* Yield 88%, mp 256–258 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.58 (s, 1H, NH), 8.34 (s, 1H, arom), 8.00–8.08 (m, 4H, arom), 7.95 (s, 1H, =CH), 7.61–7.67 (m, 2H, arom), 7.41 (d, 2H, J = 8.3 Hz, arom), 7.04 (d, 2H, J = 8.3 Hz, arom), 5.78 (dd, 1H, $\underline{CH_2}CH$, J = 11.1, 6.5 Hz), 4.12 (dd, 1H, $\underline{CH_2}CH$, J = 18.1, 11.1 Hz), 3.79 (s, 3H, OCH_3), 3.63 (dd, 1H, $CH_2\underline{CH}$, J = 18.1, 6.5 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 186.9 (C=S), 172.5 (C=O), 160.4, 159.8 (C=N), 144.3, 137.0, 134.2, 132.7, 131.5, 129.2, 128.9, 128.8, 128.7, 128.1, 128.0, 127.5, 127.2, 123.4, 114.9, 105.0 (=CH), 65.4 ($\underline{C}HCH_2$), 55.3 (OCH_3), 41.3 ($CH\underline{C}H_2$). LCMS (ESI+) m/z 446 $(M+H)^+$. Calcd. for $C_{24}H_{19}N_3O_2S_2$: C, 64.70; H, 4.30; N, 9.43; Found: C, 64.47; H, 4.58; N, 9.21%.

*4.2.3. Synthesis of [(3-methyl-4-oxo-2-thioxothiazolidin-5-ylidenemethyl)-amino]-acetic acid (**8**)*

A mixture of 5-ethoxymethylene-3-methyl-2-thioxothiazolidin-4-one **1b** (10 mmol) and glycine (12 mmol) was refluxed in 20 ml of glacial acetic acid during 3 h. The crystalline product was separated by filtration, washed with ethanol, and dried. Recrystallization from acetic acid rendered the desired product in pure form.

Yield 75%, mp 222–224 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 13.01 (brs, 1H, COOH), 8.93–8.97 (m, 0.10*1H, NH), 8.28–8.31 (m, 0.90*1H, NH), 7.63 (d, 0.90*1H, J = 13.3 Hz, =CH),

7.43 (d, 0.10*1H, J = 13.3 Hz, =CH), 4.01–4.06 (m, 2H, CH_2), 3.30 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.6 (C=S), 171.2 (C=O), 166.5 (C=O), 146.2, 89.6 (=CH), 49.0 (CH_2), 30.9 (CH_3). LCMS (ESI+) m/z 233 $(M+H)^+$. (*Z*)/(*E*) ratio (%) 71:29. Calcd. for $C_7H_8N_2O_3S_2$: C, 36.20; H, 3.47; N, 12.06; Found: C, 36.41; H, 3.27; N, 11.83%.

4.2.4. General procedure for synthesis of 5-{[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxo-ethylamino]-methylene}-3-methyl-2-thioxothiazolidin-4-ones (**9a–c**)

A mixture of appropriate 3,5-diaryl-4,5-dihydropyrazole (5 mmol) and DCC (5 mmol) was stirred at r.t. during 10 min in 10 ml of THF. Carboxylic acid **8** (5 mmol) was added and the reaction mixture was stirred at rt for 1 h. The mixture was then filtered and the filtrate was precipitated by the addition of water. After cooling, the solid precipitated was filtered off, washed with water and methanol and recrystallized from a DMF/ethanol 1:2 mixture.

*4.2.4.1. 5-({2-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-2-oxoethylamino}-methylene)-3-methyl-2-thioxothiazolidin-4-one (**9a**).* Yield 67%, mp 139–140 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 9.07–9.10 (m, 0.20*1H, NH), 8.40–8.42 (m, 0.80*1H, NH), 7.80 (brs, 2H, arom), 7.70 (d, 1H, J = 12.3 Hz, =CH), 7.46 (brs, 2H, arom), 7.46–7.53 (m, 3H, arom), 7.16 (d, 2H, J = 6.3 Hz, arom), 6.84 (d, 2H, J = 6.3 Hz, arom), 5.52 (dd, 1H, $\underline{CH_2}CH$, J = 11.8, 6.1 Hz), 4.52–4.62 (m, 2H, CH_2), 3.85 (dd, 1H, $\underline{CH_2}CH$, J = 16.1, 11.8 Hz), 3.75 (s, 3H, OCH_3), 3.29 (s, 3H, CH_3), 3.15 (dd, 1H, $CH_2\underline{CH}$, J = 16.1, 6.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.5 (C=S), 166.4 (C=O), 165.8 (C=O), 158.7, 155.7 (C=N), 146.7, 133.9, 130.9, 130.7, 128.9, 127.2, 127.0, 114.1, 89.4 (=CH), 59.6 ($\underline{C}HCH_2$), 55.2 (OCH_3), 49.8 (CH_2), 41.8 ($CH\underline{C}H_2$), 30.9 (CH_3). LCMS (ESI+) m/z 467 $(M+H)^+$. (*Z*)/(*E*) ratio (%) 75:25. Calcd. for $C_{23}H_{22}N_4O_3S_2$: C, 59.21; H, 4.75; N, 12.01; Found: C, 59.03; H, 4.95; N, 12.24%.

*4.2.4.2. 5-({2-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2-oxoethylamino}-methylene)-3-methyl-2-thioxothiazolidin-4-one (**9b**).* Yield 71%, mp 158–160 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 9.09–9.12 (m, 0.25*1H, NH), 8.42–8.46 (m, 0.75*1H, NH), 8.06–8.12 (m, 2H, arom), 7.89–7.93 (m, 3H, arom), 7.74 (d, 1H, J = 13.4 Hz, =CH), 7.54–7.56 (m, 2H, arom), 7.30–7.35 (m, 4H, arom), 5.64 (dd, 1H, $\underline{CH_2}CH$, J = 11.4, 4.1 Hz), 4.62–4.70 (m, 2H, CH_2), 4.01 (dd, 1H, $\underline{CH_2}CH$, J = 16.8, 11.4 Hz), 3.31 (s, 3H, CH_3), 3.26 (dd, 1H, $CH_2\underline{CH}$, J = 16.8, 4.1 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.5 (C=S), 166.4 (C=O), 166.1 (C=O), 155.8 (C=N), 146.7, 144.3, 133.9, 132.7, 132.1, 128.7, 128.6, 128.4, 128.3, 128.1, 127.9, 127.8, 127.6, 127.0, 123.3, 89.5 (=CH), 59.6 ($\underline{C}HCH_2$), 49.9 (CH_2), 41.7 ($CH\underline{C}H_2$), 30.9 (CH_3). LCMS (ESI+) m/z 521/523 $(M+H)^+$. (*Z*)/(*E*) ratio (%) 70:30. Calcd. for $C_{26}H_{21}ClN_4O_2S_2$: C, 59.93; H, 4.06; N, 10.75; Found: C, 59.68; H, 3.88; N, 10.92%.

*4.2.4.3. 5-({2-[5-(4-Fluorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2-oxoethylamino}-methylene)-3-methyl-2-thioxothiazolidin-4-one (**9c**).* Yield 75%, mp 192–194 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 9.09–9.12 (m, 0.20*1H, NH), 8.42–8.46 (m, 0.80*1H, NH), 8.06–8.12 (m, 2H, arom), 7.90–7.95 (m, 3H, arom), 7.74 (d, 1H, J = 13.4 Hz, =CH), 7.54–7.57 (m, 2H, arom), 7.30–7.35 (m, 2H, arom), 7.06–7.10 (m, 2H, arom), 5.65 (dd, 1H, $\underline{CH_2}CH$, J = 12.0, 3.5 Hz), 4.62–4.71 (m, 2H, CH_2), 4.01 (dd, 1H, $\underline{CH_2}CH$, J = 17.0, 12.0 Hz), 3.31 (s, 3H, CH_3), 3.09 (brs, 1H, $CH_2\underline{CH}$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 190.6 (C=S), 166.4 (C=O), 166.0 (C=O), 165.8 (d, J_{CF} = 200.0 Hz, 1C), 155.8 (C=N), 146.8, 144.3, 138.1, 133.8, 132.7, 131.9 (d, J_{CF} = 30.0 Hz, 2C), 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.6, 127.0, 123.3, 115.5 (d, J_{CF} = 21.0 Hz, 2C), 89.5 (=CH), 59.6 ($\underline{C}HCH_2$), 49.9 (CH_2), 41.9 ($CH\underline{C}H_2$), 30.9 (CH_3). LCMS (ESI+) m/z 505 $(M+H)^+$. (*Z*)/(*E*) ratio (%) 81:19. Calcd. for $C_{26}H_{21}FN_4O_2S_2$: C, 61.89; H, 4.19; N, 11.10; Found: C, 61.61; H, 4.43; N, 11.33%.

4.3. Anti-trypanosomal activity assay

Bloodstream form of *T. brucei gambiense* Feo strain were cultured in HMI9 medium supplemented with 10% FCS at 37 °C under an atmosphere of 5% CO_2 [33]. 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 [34]. 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.

4.4. In vitro cytotoxicity assay on mammalian cell

Cytotoxicity was evaluated by using a rat myoblast-derived cell line (L-6). Assays were performed in 96-well plates in RPMI medium containing 25 mM HEPES, pH 7.3, 10% fetal calf serum under 5% CO_2 atmosphere, at 37 °C. After trypsin treatment, L-6 cells were seeded at 5000 cells per well in 100 μl. After 24 h incubation, cells were washed and two-fold dilutions of drug were added (200 μl per well). Drug stock solutions were prepared in pure DMSO. The final DMSO concentration in the cultures remained below 1%. Control cultures were constituted of cultures treated with pure DMSO instead of drug. The cytotoxicity assay was based on the conversion of a redoxsensitive dye (resazurin) to a fluorescent product by viable cells [35]. After 5 days of 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 cell growth was calculated by comparing the fluorescence of cells maintained in the presence of drug to that of in the absence of drug. IC_{50}s were determined from the dose–response curves with drug concentrations ranging from 10 μg/ml to 10 ng/ml. IC_{50} value is the mean ± the standard deviation of three independent experiments. The dose–response curves have been fitted with two-parameter log-logistic function (eq. (1)), fixing upper limit at 100% and lower limit at 0%:

$$f(x) = \frac{1}{1 + e^{(b \times \ln(x) - \ln(e))}}, \quad (1)$$

where b is a Hill coefficient that defines the steepness of logistic curve and e is IC_{50} and corresponds to the position of curve on x-axis. The coefficients of determination are presented in Fig. 4 and have high values, confirming that chosen parsimonious model is accurate.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.103.

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