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Original article

Synthesis and biological activity evaluation of 5-pyrazoline substituted 4-thiazolidinones

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ABSTRACT

A series of novel 5-pyrazoline substituted 4-thiazolidinones have been synthesized. Target compounds were evaluated for their anticancer activity *in vitro* within DTP NCI protocol. Among the tested compounds, the derivatives **4d** and **4f** were found to be the most active, which demonstrated certain sensitivity profile toward the leukemia subpanel cell lines with GI_{50} value ranges of 2.12–4.58 μM (**4d**) and 1.64–3.20 μM (**4f**). The screening of antitrypanosomal and antiviral activities of 5-(3-naphthalen-2-yl-5-aryl-4,5-dihydropyrazol-1-yl)-thiazolidine-2,4-diones was carried out with the promising influence of the mentioned compounds on *Trypanosoma brucei*, but minimal effect on SARS coronavirus and influenza types A and B viruses.

1. Introduction

The non-condensed heterocyclic systems with thiazolidinone [1] and pyrazoline [2,3] moieties have emerged as powerful scaffolds in drug design. Among diazole-substituted 4-thiazolidinones highly active anticancer agents have been identified including inhibitors of necroptosis [4], tumor necrosis factor α [5] and tyrosine phosphatase [6]. Our previous study, based on a hybrid pharmacophore approach, allowed to establish a number of patterns in the structure–activity relationship (SAR) context for 4-thiazolidinones with a pyrazoline fragment in 2, 3 and 4 positions of the thiazolidone cycle, which possessed antitumor activity [7,8].

On the other hand, thiazolidinones and pyrazolines have occupied a unique position in the design and synthesis of novel biologically active agents that exert trypanocidal activity [9–13]. The 2-thioxo-4-thiazolidinone-3-acetic acid derivatives were identified as inhibitors of *Trypanosoma brucei* dolicholphosphate mannose synthase [11]. The 2-hydrazolyl-4-thiazolidinone-5-carboxylic acid derivatives have shown promising activity on the cruzipain protease [12]. The most promising compound in series of aryl-4-oxothiazolylhydrazones was shown to be very active at non-cytotoxic concentrations in *in vitro* assays against *Trypanosoma cruzi* cell cultures and exhibited potency comparable with the reference compounds (IC_{50} (Y strain) = 0.3 μM) [9]. Among pyrazoline derivatives, some novel compounds have been identified as inhibitors of the trypanosomal cysteine protease cruzain with IC_{50} of 40–230 nM [13].

The antiviral activity of heteryl substituted 4-thiazolidinones is promising. Among thiazole–thiazolidine conjugates [14] and non-condensed derivatives with thiazolidinone and pyridine [15–17] or pyrimidine [18–20] cycles, anti-HIV agents were identified. In addition, this group of compounds was active against hepatitis C virus [21], Tobacco Mosaic virus [22] and Vesicular stomatitis virus [23]. Previously we also demonstrated the efficiency of certain pyrazoline–thiazolidinone conjugates on influenza viruses and SARS coronavirus [24].

The present work is an extension of our ongoing efforts toward developing promising biologically active agents using a hybrid pharmacophore approach. We made the design (Fig. 1) and synthesized hybrid compounds by linking the main structural unit of the 4-thiazolidinone ring system with the pyrazoline, and

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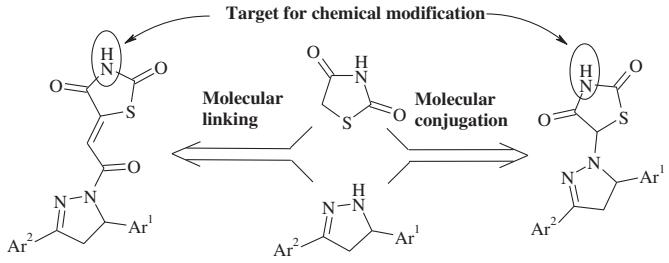


Fig. 1. Design of the non-condensed systems with the thiazolidinone and pyrazoline fragments.

examined their antitumor, trypanocidal and antiviral activities *in vitro*. We have found two compounds from 5-pyrazoline substituted 4-thiazolidinones, which possessed a commensurate antitumor activity compared to the pyrazoline–thiazolidinone analogous compounds reported previously [7,8] and evaluated anti-trypanosomal activity and antiviral activity of the synthesized compounds.

2. Results and discussion

2.1. Chemistry

The general methods for synthesis of target thiazolidinone–pyrazoline conjugates are depicted in Schemes 1 and 2.

The starting 3,5-diaryl-4,5-dihydropyrazoles synthesized using known methods from appropriate chalcones [25] easily reacted with 5-bromothiazolidine-2,4-dione [26] yielding 5-(3-naphthalen-2-yl-5-aryl-4,5-dihydropyrazol-1-yl)-thiazolidine-2,4-dione **1a** and **1b**. It is known that chemical modification of the N3 position of thiazolidinone cycle has an essential influence on the antitumor activity [27,28]. Relying on these observations we utilized potassium salt of 5-(3-naphthalen-2-yl-5-aryl-4,5-dihydropyrazol-1-yl)-thiazolidine-2,4-dione, generated *in situ*, in the reactions with 2-chloro-*N*-arylacetamides. Following the mentioned reaction the new N3-substituted non-condensed thiazolidinone–pyrazoline conjugates **2a**–**2c** were synthesized. Based on the Mannich reaction of **1a** and **1b** with secondary amines the thiazolidinone analogs **3a**–**3g** were obtained (Scheme 1).

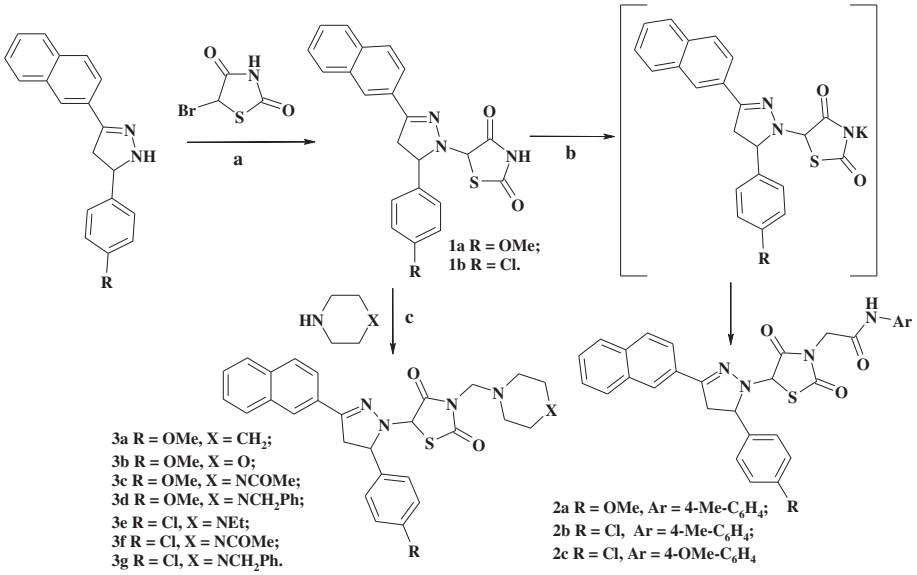
Aiming at the detailed elaboration of SAR, especially the influence of the linking group of thiazolidinone–pyrazoline conjugates on the anticancer activity, 5-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethylidene]-thiazolidine-2,4-diones (**4a**–**4f**) were synthesized by the method, described previously [29]. Reaction of 3,5-diaryl-4,5-dihydropyrazoles with (2,4-dioxothiazolidine-5-ylidene)-acetyl chloride [27] afforded in excellent yield and purity the compounds **4a**–**4f**. Following the reaction of generated *in situ* potassium salts of **4b** and **4e** with 2-chloro-*N*-arylacetamides the group of N3-substituted 4-thiazolidinones **5a**–**5e** were synthesized (Scheme 2).

The data characterization of synthesized novel heterocyclic substituted thiazolidones are presented in Experimental part. Analytical and spectral data (^{1}H NMR, ^{13}C NMR) confirmed the structure of the synthesized compounds.

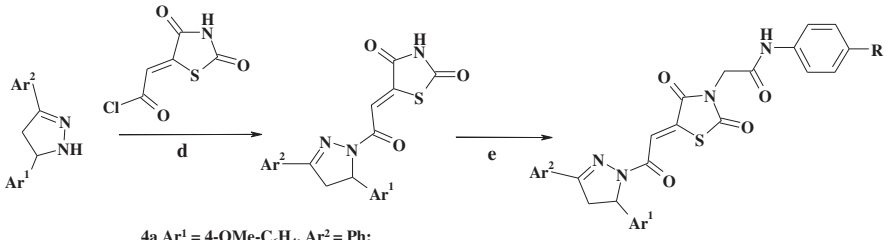
Protons CH_2–CH of pyrazoline fragment in the ^{1}H NMR spectra of synthesized compounds showed characteristic patterns of an AMX system. The proton (CH) of thiazolidinone core of **1a**–**1b**, **2a**–**2c** and **3a**–**3g** showed the broad singlet at $\delta \sim 5.59$–5.99 and the protons of the methylene group (CH_2CO) of **2a**–**2c** and **5a**–**5e** appeared as a broad singlet at $\delta \sim 4.44$–4.49 ppm. In the ^{1}H NMR spectra of the **1a**–**1b** and **4a**–**4f** NH proton of thiazolidinone cycle the broad singlet at $\delta \sim 12.20$–12.72 was found.

2.2. In vitro evaluation of the anticancer activity

Synthesized derivatives **1a**, **1b**, **2a**, **3a**–**3d**, **3f**, **4a**, **4d**, **4e**, **4f** and **5d** were selected by National Cancer Institute (NCI, Bethesda USA) Developmental Therapeutic Program (DTP) and evaluated at the concentration of 10^{-5} M toward a panel of approximately sixty cancer cell lines (http://dtp.nci.nih.gov). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, central nervous system, ovarian, renal, prostate and breast cancers. Primary anticancer assays were performed according to the NCI protocol as described elsewhere [30–33]. The compounds were added at a single concentration and the cell cultures were incubated for 48 h. The end point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results for each compound are reported as the percent growth (GP%) of treated cells



Scheme 1. Synthesis of 5-(3-naphthalen-2-yl-5-aryl-4,5-dihydropyrazol-1-yl)-thiazolidine-2,4-diones. Reagents, conditions and yields: (a) EtOH, reflux 1 h, 78–83%; (b) KOH, EtOH, reflux 5 h, 68–79%; (b) CH_2O, EtOH, r.t. 1 h, 69–85%.



Scheme 2. Synthesis of 5-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethylidene]-thiazolidine-2,4-dione. Reagents, conditions and yields: (d) triethylamine, dioxane, heating to 70–80 °C, 15 min, 69–86%; (e) KOH, EtOH, reflux 5 h, 72–87%.

when compared to untreated control cells (Table 1). The range of percent growth shows the lowest and the highest percent growth found among the different cancer cell lines.

The most active compounds **4d** and **4f** were found to be effective against 12 and 18 cell lines, respectively, compound **4a** was found to be moderately effective against few cell lines, while the other compounds (**1a**, **1b**, **2a**, **3a**–**3d**, **3f**, **4e**, **5d**) did not show any activity (Table 1).

Finally, compounds **4d** and **4f** were selected for an advanced assay against a panel of approximately sixty tumor cell lines at 10-fold dilutions of five concentrations (100 μM, 10 μM, 1 μM, 0.1 μM and 0.01 μM) [30–33]. The percentage of growth was evaluated spectrophotometrically versus controls not treated with test agents after 48-h exposure and using SRB protein assay to estimate cell viability or growth. Dose–response parameters were calculated for each cell line: GI_{50} – molar concentration of the compound that inhibits 50% net cell growth; and TGI – molar concentration of the compound leading to the total inhibition. Furthermore, a mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an average activity parameter over all cell lines for the tested compound. For the MG_MID calculation, insensitive cell lines were included with the highest concentration tested (Table 2).

The tested compounds showed inhibition activity ($GI_{50} < 10$ μM) against 47 from 55 (**4d**) and 56 from 59 (**4f**) human tumor cells with average GI_{50}/TGI values of 7.02 μM/38.07 μM (**4d**) and 4.38 μM/50.99 μM (**4f**) (Table 2). With regard to the sensitivity against some individual cell lines among several subpanel, the compounds **4d** and **4f** demonstrated a certain sensitivity profile toward the leukemia subpanel tumor cell lines with GI_{50} values range of 2.12–4.58 μM (**4d**) and 1.64–3.20 μM (**4f**) (Table 3).

The SAR study revealed that: (1) the level of antitumor activity of active thiazolidinones with pyrazoline fragment in 5 position (**4d** and **4f**) is compatible with effectivity levels of heteryl substituted thiazolidones, described previously [34–42]; (2) conjugation of pyrazoline and thiazolidinone cycles using oxomethylidene linking group (**4f**) allowed us to increase the activity, in comparison with the structurally related conjugate representative 5-(3-naphthalen-2-yl-5-aryl-4,5-dihydropyrazol-1-yl)-thiazolidine-2,4-dione **1b**; (3) introduction of the substituents in 3*N*-position of thiazolidine fragment did not have significant influence on the antitumor activity.

2.3. COMPARE analysis

NCI's COMPARE algorithm [30–33] allows to assume biochemical mechanisms of action of the novel compounds on the basis of their *in vitro* activity profiles when comparing with those of standard agents. We performed COMPARE computations for the compounds **4d** and **4f** against the NCI "Standard Agents" database at the GI_{50} and TGI levels (Table 4). However, obtained Pearson correlation coefficients (PCC) did not allow to distinguish cytotoxicity mechanism of tested compounds with high probability. The compound **4d** showed the highest correlation at the GI_{50} level with dihydroorotate dehydrogenase inhibitor brequinar (PCC = 0.651) and compound **4f** – with maytansine (RNA/DNA antimetabolite, PCC = 0.636).

2.4. Evaluation of antiviral activity

Antiviral activity of **1a**, **1b**, **2a**–**2c**, **3d** and **3e** was determined against SARS coronavirus (SARS CoV) and influenza types A and B viruses (Flu A, Flu B). The obtained results are summarized in Table 5.

Although antiviral activity was evident, virus inhibition occurred at or near the cytotoxic concentration. The compounds showed insignificant activities against the four strains of influenza virus with the range levels of selectivity index from 1.0 to 2.1. Compound **2a** had moderate activity against the duck strain of influenza A with a 50% effective concentration (EC_{50}) of 21.78 μM and selective index (SI) of >16.3; but did not have significant activity against other influenza strains. The majority of the compounds showed no activity against SARS CoV. The positive control compounds ribavirin, oseltamivir carboxylate, and M128533 were active as expected in the assays.

2.5. In vitro evaluation of antitrypanosomal activity

The compounds **1a**, **2b** and **2c** were selected in advanced *in vitro* assay against *Trypanosoma brucei brucei* (Tbb) and *Trypanosoma brucei gambiense* (Tbg). The dose–response curves with drug concentrations ranging from 10 μg/ml to 0.625 μg/ml are depicted on Fig. 2.

The results showed a moderated activity of compounds (Table 6) on both parasite strains, namely IC_{50} (Tbb) = 5.43–13.87 μM and IC_{50} (Tbg) = 2.53–6.66 μM.

3. Conclusions

In the present paper new 4-thiazolidinone based conjugates with pyrazoline moiety at 5 position are described. Antitumor activity assay of thirteen synthesized compounds allowed us to identify highly active thiazolidinone–pyrazoline hybrids **4d** and **4f**, which demonstrated certain sensitivity profile toward the leukemia subpanel tumor cell lines with GI_{50} values range of 2.12–4.58 μM (**4d**)

Table 1
Anticancer screening data at the concentration of 10 μM.

Comp	60 Cell lines assay in 1 dose 10 μM concentration			
	Mean growth %	Range of growth %	The most sensitive cell lines	Growth % of the most sensitive cell lines
1a	101.55	77.50–113.87	UO-31 (Renal Cancer)	77.50
1b	104.84	74.89–169.65	HOP-92 (Non-Small Cell Lung Cancer)	74.89
2a	99.23	76.58–113.53	UO-31 (Renal Cancer)	76.58
3a	100.94	69.54 to 112.74	HOP-92 (Non-Small Cell Lung Cancer)	69.54
3b	99.49	67.47–122.33	OVCAR-4 (Ovarian Cancer)	67.47
3c	100.67	82.16–126.20	UO-31 (Renal Cancer)	82.16
3d	102.96	74.48–127.35	UO-31 (Renal Cancer)	74.48
3f	102.12	76.37–131.30	K-562 (Leukemia)	76.37
4a	96.64	39.09–147.38	SR (Leukemia)	39.09
			K-562 (Leukemia)	58.20
			RPMI-8226 (Leukemia)	58.85
			LOX IMVI (Melanoma):	55.70
4d	60.11	−27.33–160.47	CCRF-CEM (Leukemia)	19.50
			HL-60(TB) (Leukemia)	−27.33
			K-562 (Leukemia)	33.24
			MOLT-4 (Leukemia)	16.47
			HOP-92 (Non-Small Cell Lung Cancer)	37.75
			KM12 (Colon Cancer)	29.89
			SF-295 (CNS Cancer)	−13.37
			OVCAR-3 (Ovarian Cancer)	36.41
			RXF 393 (Renal Cancer)	31.39
			PC-3 (Prostate Cancer)	33.62
			MCF7 (Breast Cancer)	30.34
			T-47D (Breast Cancer)	37.99
4e	99.73	78.68–132.27	T-47D (Breast Cancer)	78.68
4f	51.05	−5.27–109.26	CCRF-CEM (Leukemia)	16.67
			HL-60(TB) (Leukemia)	13.51
			K-562 (Leukemia)	24.64
			MOLT-4 (Leukemia)	11.47
			RPMI-8226 (Leukemia)	2.48
			SR (Leukemia)	10.57
			NCI-H522 (Non-Small Cell Lung Cancer)	23.21
			A549/ATCC (Non-Small Cell Lung Cancer)	30.04
			NCI-H460 (Non-Small Cell Lung Cancer)	25.93
			KM12 (Colon Cancer)	25.76
			SF-295 (CNS Cancer)	−5.27
			UACC-62 (Melanoma)	32.77
			MDA-MB-435 (Melanoma)	39.46
			PC-3 (Prostate Cancer)	35.99
			MCF7 (Breast Cancer)	34.41
			HS 578T (Breast Cancer)	35.58
			BT-549 (Breast Cancer)	34.25
			T-47D	39.30
5d	97.97	74.66–118.66	CCRF-CEM (Leukemia)	74.66

and 1.64–3.20 μM (**4f**). The antitrypanosomal and antiviral activities screening of 5-(3-naphthalen-2-yl-5-aryl-4,5-dihydropyrazol-1-yl)-thiazolidine-2,4-diones was carried out and demonstrated the promising influence of mentioned compounds on *T. brucei*, and no activity to minimal effect on SARS coronavirus and influenza types A and B viruses. Further investigations of such thiazolidinone derivatives could be interesting with the hope to get more selective anticancer, antiviral and antiprotozoal agents among thiazolidinone–pyrazoline hybrid analogs.

Table 3
The influence of compounds **4d** and **4f** on the growth of individual tumor cell lines ($GI_{50} < 5$ μM).

Compound	Disease	Cell line	GI_{50}, μM	TGI, μM
4d	Leukemia	CCRF-CEM	2.85	11.3
	Leukemia	RPMI-8226	2.12	6.91
	Leukemia	SR	2.85	9.02
	Leukemia	HL-60(TB)	3.35	9.14
	Leukemia	K-562	4.58	100.0
	NSC lung cancer	HOP-62	4.85	19.8
	NSC lung cancer	NCI-H460	4.18	18.7
	Colon cancer	HCT-116	4.56	16.4
	Colon cancer	HCT-15	4.85	24.6
	Colon cancer	KM12	4.76	15.3
	CNS cancer	SF-295	2.99	10.0
	CNS cancer	U251	4.29	16.6
	Melanoma	LOX IMVI	3.21	17.9
	Melanoma	MDA-MB-435	3.91	31.7
	Melanoma	UACC-62	2.83	9.28
	Ovarian cancer	OVCAR-3	3.17	11.0
	Renal cancer	786-0	2.96	13.5
	Prostate Cancer	PC-3	4.63	44.4
	Prostate Cancer	DU-145	4.64	18.4
4f	Leukemia	HL-60(TB)	3.20	6.48
	Leukemia	K-562	1.90	11.2
	Leukemia	MOLT-4	1.64	7.41
	Leukemia	RPMI-8226	1.97	5.88
	Leukemia	SR	1.98	5.92
	NSC lung cancer	A549/ATCC	3.06	100.0
	NSC lung cancer	HOP-92	1.17	9.72
	NSC lung cancer	NCI-H322M	2.89	100.0
	NSC lung cancer	NCI-H460	1.89	4.70
	Colon cancer	HCT-116	3.01	13.9
	Colon cancer	HCT-15	2.41	100.0
	CNS cancer	SF-295	2.45	9.12
	CNS cancer	SF-539	2.43	5.79
	CNS cancer	U251	2.58	13.2
	Melanoma	MDA-MB-435	2.70	16.5
	Melanoma	SK-MEL-5	2.09	22.5
	Melanoma	UACC-62	2.66	12.8
	Ovarian cancer	OVCAR-3	2.90	10.6
	Renal cancer	A498	1.41	5.38
	Prostate Cancer	PC-3	4.94	100.0
	Prostate Cancer	DU-145	4.27	100.0
	Breast cancer	MCF-7	2.53	14.1
	Breast cancer	HS 578T	1.94	8.26
	Breast cancer	BT-549	2.16	7.75
	Breast cancer	T-47D	1.53	9.40

4. Experimental

4.1. Materials and methods

The starting 3,5-diaryl-4,5-dihydro-1*H*-pyrazole [25], 5-bromothiazolidine-2,4-dione [26], and (2,4-dioxothiazolidine-5-ylidene)-acetyl chloride [27] were obtained according to the

Table 2
Anticancer activity against a panel of approximately sixty tumor cell lines from nine different cancer types at 10-fold dilutions of five concentrations.

Compound	End point (μM)	Leukemia	NSC lung cancer	Colon cancer	CNS cancer	Melanoma	Ovarian cancer	Renal cancer	Prostate cancer	Breast cancer	MG_MID
4d	GI_{50}	3.28	7.91	5.73	7.57	8.07	11.37	8.13	4.64	6.50	7.02
	TGI	25.31	42.06	21.01	24.10	28.21	79.20	44.04	31.40	47.30	38.07
4f	GI_{50}	2.14	3.52	3.53	4.09	4.08	9.79	4.66	4.61	2.99	4.38
	TGI	7.38	61.59	63.20	31.99	45.88	79.83	53.25	100.0	15.85	50.99

Table 4
COMPARE analysis results for compounds **4d** and **4f**.

Compound	End point	PCC[a]	Target	Target vector NSC	Target mechanism of action[b]
4d	GI_{50}	0.651	Brequinar	S368390	Dihydroorotate dehydrogenase inhibitor
		0.634	Dichloroallyl lawsone	S126771	DNA/RNA antimetabolite
		0.626	Trimetrexate	S352122	Dihydrofolate reductase inhibitor
		0.62	L-Cysteine analog	S303861	Reversible binding inhibitor of the human kinesin Eg5, antimitotic agent
		0.586	Soluble Baker's Antifol	S139105	RNA/DNA antimetabolite
		0.568	Glycoxalic acid	S267213	
4f	GI_{50}	0.636	Maytansine	S153858	RNA/DNA antimetabolite
		0.573	Rhizoxin	S749069	Microtubule polymerization inhibitor
		0.550	Macbecin II	S269148	DNA antimetabolite

[a] Only correlations with PCC $\geq$ 0.55 were selected, as significant.
[b] Putative mechanisms of action were identified with the use of literature sources.

methods described previously. Preparation of compounds **4a**–**4d** and **4f** was described in our previous report [29].

Melting points were measured in open capillary tubes on a BŰCHI B-545 melting point apparatus and are uncorrected. The elemental analyses (C, H, N) were performed using the 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

Table 5
Antiviral activity of the synthesized compounds.

Compound	Virus	Virus strain	EC_{50}[a] μM	SD[b]	CC_{50}[c] μM	SD	SI[d]
1a	Flu A (H1N1)	California/07/2009	9.82	6.23	10.30	5.75	1.1
	Flu A (H3N2)	Perth/16/2009	4.31	3.35	6.23	4.55	1.4
	Flu A (H5N1)	Duck/MN/1525/81	7.90	0.45	12.46	6.95	1.6
	Flu B	Florida/4/2006	4.55	2.63	8.38	5.75	1.8
	SARS CoV	Urbani	>23.47	6.95	23.47	6.95	0
1b	Flu A (H1N1)	California/07/2009	4.27	3.08	4.50	3.32	1.0
	Flu A (H3N2)	Perth/16/2009	2.84	0.71	3.08	0.71	1.0
	Flu A (H5N1)	Duck/MN/1525/81	5.21	2.13	6.40	2.61	1.2
	Flu B	Florida/4/2006	3.08	1.66	4.98	2.13	1.6
	SARS CoV	Urbani	>23.23	6.87	23.23	6.87	0
2a	Flu A (H1N1)	California/07/2009	141.66	40.73	219.60	90.32	1.6
	Flu A (H3N2)	Perth/16/2009	53.13	53.13	74.38	88.55	1.4
	Flu A (H5N1)	Duck/MN/1525/81	21.78	20.01	>354.19	0	>16.3
	Flu B	Florida/4/2006	79.69	70.84	170.01	141.68	2.1
	SARS CoV	Urbani	>313.46	70.84	313.46	70.84	0
2b	Flu A (H1N1)	California/07/2009	54.47	24.60	73.80	22.84	1.3
	Flu A (H3N2)	Perth/16/2009	>13.35	6.68	13.35	6.68	0
	Flu A (H5N1)	Duck/MN/1525/81	50.96	33.39	56.23	28.12	1.1
	Flu B	Florida/4/2006	35.14	36.90	42.17	35.14	1.3
	SARS CoV	Urbani	>351.44	0	>351.44	0	0
2c	Flu A (H1N1)	California/07/2009	119.64	100.84	176.04	119.64	1.5
	Flu A (H3N2)	Perth/16/2009	18.80	7.52	25.64	18.80	1.3
	Flu A (H5N1)	Duck/MN/1525/81	179.46	111.09	264.92	94.00	1.5
	Flu B	Florida/4/2006	95.71	164.08	131.60	158.95	1.4
	SARS CoV	Urbani	>341.83	0	>341.83	0	0
3d	Flu A (H1N1)	California/07/2009	13.70	16.34	14.69	15.68	1.1
	Flu A (H3N2)	Perth/16/2009	>6.60	3.63	6.60	3.63	0
	Flu A (H5N1)	Duck/MN/1525/81	>24.76	24.76	24.76	24.76	0
	Flu B	Florida/4/2006	57.78	75.94	59.43	75.94	1.0
	SARS CoV	Urbani	21.46	5.78	34.67	19.81	1.6
3e	Flu A (H1N1)	California/07/2009	4.01	1.82	4.56	2.37	1.1
	Flu A (H3N2)	Perth/16/2009	>2.37	0.73	2.37	0.73	0
	Flu A (H5N1)	Duck/MN/1525/81	4.38	2.19	6.20	4.74	1.4
	Flu B	Florida/4/2006	4.01	4.20	8.39	10.22	2.1
	SARS CoV	Urbani	>16.78	3.47	16.78	3.47	0
Ribavirin	Flu A (H1N1)	California/07/2009	23.75	2.46	>348.07	122.85	>15
	Flu A (H3N2)	Perth/16/2009	18.43	11.88	>368.54	69.61	>20
	Flu A (H5N1)	Duck/MN/1525/81	18.43	7.37	>323.50	90.09	>20
	Flu B	Florida/4/2006	5.73	2.05	>405.39	12.28	>69
Oseltamirvir-carboxylate	Flu A (H1N1)	California/07/2009	18.99	10.90	>35.17	0.0	>1.9
	Flu A (H3N2)	Perth/16/2009	3.06	2.81	>35.17	0.0	>11
	Flu A (H5N1)	Duck/MN/1525/81	0.11	0.007	>35.17	0.0	>326
	Flu B	Florida/4/2006	3.52	4.22	>35.17	0.0	>10
M128533 Protease inhibitor	SARS CoV	Urbani	1.56	1.18	>236.41	0.0	>152

[a] 50% Effective (virus-inhibitory) concentration.
[b] 50% Cytotoxic concentration, determined in uninfected cells.
[c] SD – standard deviation.
[d] Selectivity index (CC_{50}/EC_{50}), derived from three independent experiments.

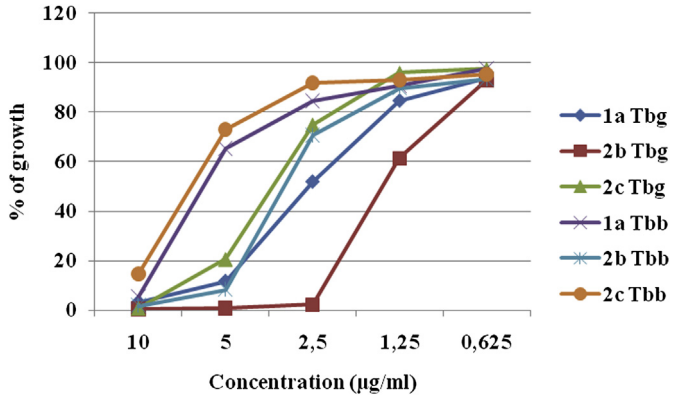


Fig. 2. The dose–response curves of compounds **1a**, **2b** and **2c** on *Trypanosoma brucei brucei* (Tbb) and *Trypanosoma brucei gambiense* (Tbg) growth.

standard. Chemical shifts are reported in ppm units with use of δ scale.

4.2. Chemistry

4.2.1. General procedure for synthesis of 5-(3-naphthalen-2-yl-5-aryl-4,5-dihydropyrazol-1-yl)-thiazolidine-2,4-diones (**1a**, **1b**)

A mixture of 50 mmol 5-bromothiazolidine-2,4-dione and 50 mmol of appropriate 3,5-diaryl-4,5-dihydropyrazole was refluxed in 100 ml of ethanol during 1 h. The crystalline products were separated by filtration, washed with ethanol, and dried. Recrystallization from acetic acid rendered desired products in pure form.

*4.2.1.1. 5-[5-(4-Methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-thiazolidine-2,4-dione (**1a**).* Yield 78%, mp 208–210 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.20 (s, 1H, NH), 8.08 (s, 1H, arom), 7.87–7.97 (m, 4H, arom), 7.55–7.58 (m, 2H, arom), 7.50 (d, 2H, J = 8.4 Hz, arom), 7.02 (d, 2H, J = 8.4 Hz, arom), 5.74 (s, 1H, CH, thiazol), 4.29 (dd, 1H, $\underline{CH_2}CH$, J = 13.5, 10.1 Hz), 3.83 (dd, 1H, $\underline{CH_2}CH$, J = 16.8, 10.1 Hz), 3.78 (s, 3H, OCH_3), 3.16 (dd, 1H, $CH_2\underline{CH}$, J = 16.8, 13.5 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.6 (C=O), 171.7 (C=O), 159.9, 154.7 (C=N), 133.9, 133.3, 130.1, 130.0, 129.6, 128.9, 128.7, 128.2, 127.5, 127.2, 127.0, 123.5, 114.8, 70.1 (CH), 66.1 ($\underline{CH}CH_2$), 55.7 (OCH_3), 42.2 ($CH\underline{CH_2}$). Calcd. for $C_{23}H_{19}N_3O_3S$: C, 66.17; H, 4.59; N, 10.06; Found: C, 66.38; H, 4.77; N, 10.28%.

*4.2.1.2. 5-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-thiazolidine-2,4-dione (**1b**).* Yield 83%, mp 218–220 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.21 (s, 1H, NH), 8.06 (s, 1H, arom), 7.81–7.97 (m, 4H, arom), 7.49–7.62 (m, 5H, arom), 7.42 (s, 1H, arom), 5.79 (s, 1H, CH, thiazol), 4.41 (dd, 1H, $\underline{CH_2}CH$, J = 13.5, 10.3 Hz), 3.82 (dd, 1H, $\underline{CH_2}CH$, J = 16.5, 10.3 Hz), 3.14 (dd, 1H, $CH_2\underline{CH}$, J = 16.5, 13.5 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.5 (C=O), 171.6 (C=O), 154.6 (C=N), 137.9, 133.9, 133.4, 133.2, 130.1, 129.3, 129.0, 128.9, 128.7, 128.2, 127.5, 127.2, 127.1, 123.6, 74.0 (CH), 69.4 ($\underline{CH}CH_2$), 42.5 ($CH\underline{CH_2}$). Calcd. for $C_{22}H_{16}ClN_3O_2S$: C, 62.63; H, 3.82; N, 9.96; Found: C, 62.48; H, 3.98; N, 9.75%.

Table 6
Anti-trypanosomal activity of 5-(3-naphthalen-2-yl-5-aryl-4,5-dihydropyrazol-1-yl)-thiazolidine-2,4-dione derivatives (**1a**, **2b**, **2c**).

Comp	Trypanosoma B.B.		Trypanosoma B.G.	
	IC_{50}, μM	SD	IC_{50}, μM	SD
1a	13.87	0.36	6.66	1.15
2b	5.43	0.09	2.53	0.12
2c	11.26	0.31	6.10	0.43
Pentamidine	0.0032	0.0003	0.0053	0.0009

IC_{50} value is the mean +/− the standard deviation (SD) of three independent experiments.

4.2.2. General procedure for synthesis of 2-{5-[5-aryl-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2,4-dioxothiazolidin-3-yl}-N-arylacetamides (**2a**–**2c**)

A suspension of compound **1a** or **1b** (3 mmol) and potassium hydroxide (3 mmol) was stirred at r.t. during 5 min, later appropriate 2-chloro-*N*-arylacetamide (3.3 mmol) was added and the mixture was refluxed for 5 h in EtOH (10 ml). Obtained powders were filtered off, washed with ethanol and recrystallized with DMF:ethanol (1:2) mixtures.

*4.2.2.1. 2-{5-[5-(4-Methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2,4-dioxothiazolidin-3-yl}-N-p-tolylacetamide (**2a**).* Yield 73%, mp 216–218 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 10.03 (s, 1H, NH), 8.14 (s, 1H, arom), 7.87–7.97 (m, 4H, arom), 7.55–7.58 (m, 2H, arom), 7.52 (d, 2H, J = 8.6 Hz, arom), 7.30 (d, 2H, J = 8.3 Hz, arom), 7.17 (d, 2H, J = 8.3 Hz, arom), 7.03 (d, 2H, J = 8.6 Hz, arom), 5.91 (s, 1H, CH, thiazol), 4.45 (br. s, 2H, CH_2), 4.40 (dd, 1H, $\underline{CH_2}CH$, J = 13.8, 10.5 Hz), 3.83 (dd, 1H, $\underline{CH_2}CH$, J = 16.1, 10.5 Hz), 3.79 (s, 3H, OCH_3), 3.19 (dd, 1H, $CH_2\underline{CH}$, J = 16.1, 13.8 Hz), 2.28 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.6 (C=O), 171.7 (C=O), 163.8 (C=O), 159.9, 154.7 (C=N), 133.9, 133.3, 132.7, 131.5, 130.8, 130.1, 130.0, 129.7, 129.6, 129.4, 128.7, 128.2, 127.5, 127.2, 127.0, 123.5, 114.8, 70.1 (CH), 66.1 ($\underline{CH}CH_2$), 55.7 (OCH_3), 44.1 (CH_2), 42.2 ($CH\underline{CH_2}$), 20.9 (CH_3). Calcd. for $C_{32}H_{28}N_4O_4S$: C, 68.07; H, 5.00; N, 9.92; Found: C, 67.88; H, 5.15; N, 9.68%.

*4.2.2.2. 2-{5-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2,4-dioxothiazolidin-3-yl}-N-p-tolylacetamide (**2b**).* Yield 68%, mp 220–222 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 10.05 (s, 1H, NH), 7.99 (s, 1H, arom), 7.83–7.89 (m, 3H, arom), 7.63 (d, 2H, J = 8.3 Hz, arom), 7.43–7.50 (m, 4H, arom), 7.07 (d, 2H, J = 8.3 Hz, arom), 5.81 (s, 1H, CH, thiazol), 4.52 (dd, 1H, $\underline{CH_2}CH$, J = 13.8, 9.9 Hz), 4.47 (br. s, 2H, CH_2), 3.85 (dd, 1H, $\underline{CH_2}CH$, J = 16.2, 9.9 Hz), 3.16 (dd, 1H, $CH_2\underline{CH}$, J = 16.8, 13.8 Hz), 2.31 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.5 (C=O), 171.6 (C=O), 164.0 (C=O), 154.6 (C=N), 137.9, 133.9, 133.4, 133.2, 132.7, 131.5, 130.8, 130.1, 129.7, 129.3, 129.0, 128.9, 128.7, 128.2, 127.5, 127.2, 127.1, 123.6, 74.0 (CH), 69.4 ($\underline{CH}CH_2$), 44.1 (CH_2), 42.5 ($CH\underline{CH_2}$), 21.0 (CH_3). Calcd. for $C_{31}H_{25}ClN_4O_3S$: C, 65.43; H, 4.43; N, 9.85; Found: C, 65.62; H, 4.61; N, 9.68%.

*4.2.2.3. 2-{5-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2,4-dioxothiazolidin-3-yl}-N-(4-methoxyphenyl)-acetamide (**2c**).* Yield 79%, mp 212–214 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 10.21 (s, 1H, NH), 8.10 (s, 1H, arom), 7.88–7.95 (m, 4H, arom), 7.49–7.63 (m, 8H, arom), 6.93 (d, 2H, J = 8.6 Hz, arom), 5.99 (s, 1H, CH, thiazol), 4.50 (dd, 1H, $\underline{CH_2}CH$, J = 13.3, 9.7 Hz), 4.45 (br. s, 2H, CH_2), 3.85 (dd, 1H, $\underline{CH_2}CH$, J = 16.8, 9.7 Hz), 3.72 (s, 3H, OCH_3), 3.16 (dd, 1H, $CH_2\underline{CH}$, J = 16.8, 13.3 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.8 (C=O), 170.6 (C=O), 163.6 (C=O), 155.9, 154.6 (C=N), 138.0, 133.9, 133.3, 133.2, 132.2, 130.1, 129.3, 129.0, 128.8, 128.6, 128.2, 127.5, 127.2, 127.1, 123.9, 121.2, 114.4, 72.6 (CH), 69.2 ($\underline{CH}CH_2$), 55.7 (OCH_3), 44.3 (CH_2), 42.6 ($CH\underline{CH_2}$). Calcd. for $C_{31}H_{25}ClN_4O_4S$: C, 63.64; H, 4.31; N, 9.58; Found: C, 63.79; H, 4.55; N, 9.71%.

4.2.3. General procedure for synthesis of 5-[5-aryl-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-3-R-methylthiazolidine-2,4-diones (**3a**–**3g**)

A mixture of compound **1a** or **1b** (3 mmol), appropriate amine (3.3 mmol) and formaldehyde (3 mmol) was stirred at r.t. during 1 h

in EtOH (10 ml). Obtained powders were filtered off, washed with ethanol and recrystallized with DMF:ethanol (1:2) mixtures.

*4.2.3.1. 5-[5-(4-Methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-3-piperidin-1-ylmethylthiazolidine-2,4-dione (**3a**).* Yield 75%, mp 202–204 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.94–7.97 (m, 2H, arom), 7.76–7.83 (m, 3H, arom), 7.49–7.51 (m, 4H, arom), 6.97 (d, 2H, J = 7.7 Hz, arom), 5.54 (s, 1H, CH, thiazol), 4.59 (br. s, 2H, NCH_2N), 4.34 (dd, 1H, $\underline{CH_2}CH$, J = 12.6, 9.9 Hz), 3.83 (s, 3H, OCH_3), 3.75 (dd, 1H, $\underline{CH_2}CH$, J = 16.4, 9.9 Hz), 3.15 (dd, 1H, CH_2CH, J = 16.4, 12.6 Hz), 2.67–2.72 (m, 4H, 2*CH_2), 1.52–1.57 (m, 6H, 3*CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.8 (C═O), 170.6 (C═O), 159.9, 154.6 (C═N), 133.9, 133.3, 130.0, 129.6, 129.5, 128.8, 128.4, 128.2, 127.5, 127.3, 127.2, 123.9, 114.7, 72.6 (CH), 70.0 ($\underline{C}HCH_2$), 55.7 (OCH_3), 51.7 (CH_2), 42.2 ($CH\underline{C}H_2$), 40.9, 40.8, 26.1. Calcd. for $C_{29}H_{30}N_4O_3S$: C, 67.68; H, 5.88; N, 10.89; Found: C, 67.49; H, 5.62; N, 10.72%.

*4.2.3.2. 5-[5-(4-Methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-3-morpholin-4-ylmethylthiazolidine-2,4-dione (**3b**).* Yield 85%, mp 195–196 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.13 (s, 1H, arom), 7.92–7.97 (m, 4H, arom), 7.52–7.56 (m, 2H, arom), 7.34 (d, 2H, J = 8.1 Hz, arom), 6.94 (d, 2H, J = 8.1 Hz, arom), 5.74 (s, 1H, CH, thiazol), 5.09 (br. s, 2H, NCH_2N), 4.33 (dd, 1H, $\underline{CH_2}CH$, J = 13.1, 9.7 Hz), 3.74–3.81 (m, 4H), 3.51–3.54 (m, 4H, 2*CH_2) 3.38 (dd, 1H, $CH_2\underline{CH}$, J = 16.7, 13.1 Hz), 2.45–2.47 (m, 4H, 2*CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.5 (C═O), 171.5 (C═O), 159.1, 153.6 (C═N), 133.8, 133.4, 130.1, 129.6, 129.5, 128.8, 128.6, 128.2, 127.5, 127.3, 127.1, 123.8, 114.8, 70.9 (CH), 69.6 ($\underline{C}HCH_2$), 66.6, 62.9, 55.6 (OCH_3), 50.7 (CH_2), 41.0 ($CH\underline{C}H_2$). Calcd. for $C_{28}H_{28}N_4O_4S$: C, 65.10; H, 5.46; N, 10.84; Found: C, 65.32; H, 5.69; N, 10.69%.

*4.2.3.3. 3-(4-Acetylpiperazin-1-ylmethyl)-5-[5-(4-methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydro-pyrazol-1-yl]-thiazolidine-2,4-dione (**3c**).* Yield 79%, mp 185–187 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 7.84–7.88 (m, 5H, arom), 7.49–7.51 (m, 4H, arom), 6.97 (d, 2H, J = 7.6 Hz, arom), 5.59 (s, 1H, CH, thiazol), 4.70 (d, 1H, J = 12.9 Hz, NCH_2N), 4.65 (d, 1H, J = 12.9 Hz, NCH_2N), 4.33 (dd, 1H, $\underline{CH_2}CH$, J = 12.9, 9.7 Hz), 3.73–3.83 (m, 4H), 3.44–3.46 (m, 4H, 2*CH_2) 3.15 (dd, 1H, $CH_2\underline{CH}$, J = 15.8, 12.9 Hz), 2.69–2.80 (m, 4H, 2*CH_2), 1.99 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.6 (C═O), 171.7 (C═O), 168.8 (C═O), 159.9, 154.7 (C═N), 133.9, 133.3, 130.1, 130.0, 129.6, 128.9, 128.7, 128.2, 127.5, 127.2, 127.0, 123.5, 114.8, 70.1 (CH), 66.1 ($\underline{C}HCH_2$), 63.0, 55.7 (OCH_3), 51.0, 50.1, 46.1, 42.2 ($CH\underline{C}H_2$), 41.2. Calcd. for $C_{30}H_{31}N_5O_4S$: C, 64.61; H, 5.60; N, 12.56; Found: C, 64.78; H, 5.46; N, 12.40%.

*4.2.3.4. 3-(4-Benzylpiperazin-1-ylmethyl)-5-[5-(4-methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-thiazolidine-2,4-dione (**3d**).* Yield 80%, mp 168–170 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.09 (s, 1H, arom), 7.79–7.80 (m, 4H, arom), 7.50–7.55 (m, 4H, arom), 7.19–7.28 (m, 5H, arom), 7.01 (d, 2H, J = 8.4 Hz, arom), 5.72 (s, 1H, CH, thiazol), 4.55 (br. s, 2H, NCH_2N), 4.33 (dd, 1H, $\underline{CH_2}CH$, J = 13.0, 9.8 Hz), 3.77–3.84 (m, 4H), 3.45–3.51 (m, 4H, 2*CH_2) 3.18 (dd, 1H, $CH_2\underline{CH}$, J = 15.0, 13.0 Hz), 2.65–2.69 (m, 2H, CH_2), 2.38–2.41 (m, 4H, 2*CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.2 (C═O), 172.1 (C═O), 159.9, 155.1 (C═N), 133.9, 133.3, 130.1, 130.0, 129.6, 129.5, 128.9, 128.8, 128.6, 128.5, 128.1, 127.6, 127.3, 127.2, 127.1, 123.7, 114.8, 72.5 (CH), 69.9 ($\underline{C}HCH_2$), 55.7 (OCH_3), 55.6, 50.7, 42.3 ($CH\underline{C}H_2$), 40.9. Calcd. for $C_{35}H_{35}N_5O_3S$: C, 69.40; H, 5.82; N, 11.56; Found: C, 69.23; H, 5.96; N, 11.40%.

*4.2.3.5. 5-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-3-(4-ethylpiperazin-1-ylmethyl)-thiazolidine-2,4-dione (**3e**).* Yield 72%, mp 182–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.11 (s, 1H, arom), 7.92–7.96 (m, 4H, arom), 7.54–7.61 (m, 5H, arom), 7.43 (br. s, 1H, arom), 5.78 (s, 1H, CH, thiazol), 4.58 (d, 1H, J = 12.9 Hz, NCH_2N), 4.54 (d, 1H, J = 12.9 Hz, NCH_2N), 4.42 (dd, 1H, $\underline{CH_2}CH$, J = 13.3, 10.0 Hz), 3.85 (dd, 1H, $\underline{CH_2}CH$, J = 16.0, 10.0 Hz), 3.47–3.55 (m, 4H, 2*CH_2), 3.16 (dd, 1H, $CH_2\underline{CH}$, J = 16.0, 13.3 Hz), 3.64–3.67 (m, 2H, CH_2), 2.39–2.43 (m, 4H, 2*CH_2), 1.03 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.9 (C═O), 171.7 (C═O), 154.6 (C═N), 137.9, 133.9, 133.3, 133.2, 130.1, 129.3, 128.9, 128.7, 128.6, 128.2, 127.6, 127.2, 127.1, 123.6, 74.0 (CH), 69.4 ($\underline{C}HCH_2$), 62.8, 52.3, 51.9, 42.5 ($CH\underline{C}H_2$), 40.9. Calcd. for $C_{29}H_{30}ClN_5O_2S$: C, 63.55; H, 5.52; N, 12.78; Found: C, 63.69; H, 5.38; N, 12.56%.

*4.2.3.6. 3-(4-Acetylpiperazin-1-ylmethyl)-5-[5-(4-chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-thiazolidine-2,4-dione (**3f**).* Yield 79%, mp 211–212 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.02 (s, 1H, arom), 7.78–7.93 (m, 5H, arom), 7.61 (d, 2H, J = 7.8 Hz, arom), 7.50–7.54 (m, 4H, arom), 7.43 (br. s, 1H, arom), 5.80 (s, 1H, CH, thiazol), 4.62 (br. s, 2H, NCH_2N), 4.66 (dd, 1H, $\underline{CH_2}CH$, J = 13.0, 10.2 Hz), 3.88 (dd, 1H, $\underline{CH_2}CH$, J = 15.9, 10.2 Hz), 3.44–3.55 (m, 4H, 2*CH_2), 3.16 (dd, 1H, $CH_2\underline{CH}$, J = 15.9, 13.0 Hz), 2.60–2.66 (m, 4H, 2*CH_2), 2.00 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.2 (C═O), 172.1 (C═O), 168.8 (C═O), 155.0 (C═N), 137.9, 133.9, 133.4, 133.2, 130.1, 129.3, 129.2, 128.9, 128.8, 128.2, 127.7, 127.5, 127.3, 123.2, 72.6 (CH), 69.2 ($\underline{C}HCH_2$), 63.0, 51.0, 50.1, 46.1, 42.6 ($CH\underline{C}H_2$), 41.2. Calcd. for $C_{29}H_{28}ClN_5O_3S$: C, 61.97; H, 5.02; N, 12.46; Found: C, 61.72; H, 5.21; N, 12.34%.

*4.2.3.7. 3-(4-Benzylpiperazin-1-ylmethyl)-5-[5-(4-chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-thiazolidine-2,4-dione (**3g**).* Yield 69%, mp 194–196 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 8.06 (s, 1H, arom), 7.87–7.93 (m, 5H, arom), 7.60 (d, 2H, J = 8.1 Hz, arom), 7.40–7.54 (m, 4H, arom), 7.22–7.26 (m, 4H, arom), 5.79 (s, 1H, CH, thiazol), 4.45–4.56 (m, 3H), 3.86 (dd, 1H, $\underline{CH_2}CH$, J = 16.8, 9.8 Hz), 3.47–3.54 (m, 4H, 2*CH_2), 3.28 (dd, 1H, $CH_2\underline{CH}$, J = 16.8, 13.2 Hz), 2.56–2.68 (m, 2H, CH_2), 2.32–2.41 (m, 4H, 2*CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.2 (C═O), 172.1 (C═O), 155.0 (C═N), 137.9, 133.9, 133.4, 133.2, 130.5, 130.1, 129.3, 129.2, 128.9, 128.7, 128.6, 128.5, 128.1, 127.6, 127.4, 127.3, 127.2, 123.7, 72.6 (CH), 69.3 ($\underline{C}HCH_2$), 63.0, 53.1, 50.1, 42.6 ($CH\underline{C}H_2$), 41.0. Calcd. for $C_{34}H_{32}ClN_5O_2S$: C, 66.93; H, 5.29; N, 11.48; Found: C, 66.68; H, 5.10; N, 11.22%.

*4.2.4. General procedure for synthesis of 5-[2-(3,5-diaryll-4,5-dihydropyrazol-1-yl)-2-oxoethylidene]-thiazolidine-2,4-diones (**4a**–**4f**)*

A solution of (2,4-dioxothiazolidin-5-ylidene)-acetyl chloride (3 mmol) in 5 ml of dioxane was added to a mixture of appropriate 3,5-diaryl-4,5-dihydro-1*H*-pyrazole (3 mmol) and triethylamine (3 mmol) in 5 ml of dioxane and later was heated to 70–80 °C during 15 min, cooled and poured water (50 ml). Obtained powder was filtered off, washed with water and recrystallized with DMF:ethanol (1:2) mixtures.

*4.2.4.1. 5-{2-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-thiazolidine-2,4-dione (**4a**).* Yield 75%, mp 240–242 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.64 (br. s, 1H, NH), 7.81–7.86 (m, 3H, arom), 7.48–7.51 (m, 3H, arom, CH), 7.14 (d, 2H, J = 8.6 Hz, arom), 6.87 (d, 2H, J = 8.6 Hz, arom), 5.59 (dd, 1H, $CH_2\underline{CH}$, J = 11.2, 4.3 Hz), 3.93 (dd, 1H, $\underline{CH_2}CH$, J = 18.3, 11.2 Hz), 3.70 (s, 3H, OCH_3), 3.21 (dd, 1H, $\underline{CH_2}CH$, J = 18.3, 4.3 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.3 (C═O), 167.1 (C═O), 160.9 (C═O), 159.2, 157.4 (C═N), 139.9, 133.8, 131.4, 131.0, 129.4, 127.6, 127.5, 117.8, 114.6, 60.2 ($\underline{C}HCH_2$), 55.6 (OCH_3), 42.7 ($CH\underline{C}H_2$). Calcd. for $C_{21}H_{17}N_3O_4S$: C, 61.91; H, 4.21; N, 10.31; Found: C, 61.78; H, 4.08; N, 10.13%.

*4.2.4.2. 5-{2-[5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-thiazolidine-2,4-dione (**4b**).* Yield 86%, mp 250–252 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.72 (br. s, 1H, NH), 7.84–7.87 (m, 3H, arom), 7.50–7.54 (m, 3H, arom, CH), 7.41 (d, 2H, J = 8.1 Hz, arom), 7.28 (d, 2H, J = 8.1 Hz, arom), 5.68 (dd, 1H, $CH_2\underline{CH}$, J = 11.4, 4.3 Hz), 3.97 (dd, 1H, $\underline{CH_2}CH$, J = 18.0, 11.4 Hz), 3.29 (dd, 1H, $\underline{CH_2}CH$, J = 18.0, 4.3 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.4 (C=O), 167.1 (C=O), 160.9 (C=O), 157.7 (C=N), 137.9, 133.4, 133.2, 131.2, 129.5, 129.3, 129.0, 127.4, 117.9, 116.0, 57.2 ($\underline{C}HCH_2$), 41.6 ($CH\underline{C}H_2$). Calcd. for $C_{20}H_{14}ClN_3O_3S$: C, 58.32; H, 3.43; N, 10.20; Found: C, 58.58; H, 3.28; N, 10.03%.

*4.2.4.3. 5-{2-[5-(2-Hydroxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-thiazolidine-2,4-dione (**4c**).* Yield 82%, mp 232–234 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.67 (br. s, 1H, NH), 9.72 (s, 1H, OH), 7.80–7.85 (m, 3H, arom), 7.46–7.49 (m, 3H, arom, CH), 7.07 (t, 1H, J = 7.8 Hz, arom), 6.90 (d, 1H, J = 6.7 Hz, arom), 6.80 (d, 1H, J = 7.8 Hz, arom), 6.71 (t, 1H, J = 7.4 Hz, arom), 5.72 (dd, 1H, $CH_2\underline{CH}$, J = 11.6, 4.6 Hz), 3.88 (dd, 1H, $\underline{CH_2}CH$, J = 17.9, 11.6 Hz), 3.14 (dd, 1H, $\underline{CH_2}CH$, J = 17.9, 4.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.4 (C=O), 167.1 (C=O), 160.9 (C=O), 157.7 (C=N), 154.7, 139.7, 131.2, 129.3, 128.9, 127.4, 127.0, 119.4, 117.9, 116.0, 57.2 ($\underline{C}HCH_2$), 41.6 ($CH\underline{C}H_2$). Calcd. for $C_{20}H_{15}N_3O_4S$: C, 61.06; H, 3.84; N, 10.68; Found: C, 61.27; H, 3.71; N, 10.53%.

*4.2.4.4. 5-{2-[5-(2-Hydroxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-thiazolidine-2,4-dione (**4d**).* Yield 69%, mp 245–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.57 (br. s, 1H, NH), 10.08 (s, 1H, OH), 7.82 (s, 1H, CH), 7.76 (d, 2H, J = 8.6 Hz, arom), 7.24 (d, 1H, J = 8.5 Hz, arom), 6.99–7.04 (m, 3H, arom), 6.77 (d, 1H, J = 8.6 Hz, arom), 5.64 (dd, 1H, $CH_2\underline{CH}$, J = 11.4, 4.8 Hz), 3.77–3.91 (m, 4H), 3.14 (dd, 1H, $\underline{CH_2}CH$, J = 18.2, 4.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.3 (C=O), 167.2 (C=O), 162.7 (C=O), 161.9, 160.8, 157.5 (C=N), 154.2, 139.7, 131.6, 129.8, 129.5, 129.2, 123.6, 118.3, 117.9, 114.8, 110.5, 56.8 ($\underline{C}HCH_2$), 55.9 (OCH_3), 41.4 ($CH\underline{C}H_2$). Calcd. for $C_{21}H_{17}N_3O_5S$: C, 59.57; H, 4.05; N, 9.92; Found: C, 59.73; H, 4.21; N, 9.78%.

*4.2.4.5. 5-{2-[5-(4-Methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-thiazolidine-2,4-dione (**4e**).* Yield 78%, mp 271–273 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.71 (br. s, 1H, NH), 8.27 (s, 1H, arom), 8.08 (d, 1H, J = 8.6 Hz, arom), 7.96–8.02 (m, 3H, arom), 7.87 (s, 1H, CH), 7.56–7.61 (m, 2H, arom), 7.18 (d, 2H, J = 8.3 Hz, arom), 6.89 (d, 2H, J = 8.3 Hz, arom), 5.64 (dd, 1H, $CH_2\underline{CH}$, J = 11.4, 4.0 Hz), 4.03 (dd, 1H, $\underline{CH_2}CH$, J = 18.0, 11.4 Hz), 3.72 (s, 3H, OCH_3), 3.39 (dd, 1H, $\underline{CH_2}CH$, J = 18.0, 4.0 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.4 (C=O), 167.2 (C=O), 160.9 (C=O), 159.2, 157.5 (C=N), 140.1, 134.4, 133.8, 133.2, 129.1, 128.9, 128.7, 128.6, 128.2, 128.1, 127.6, 127.4, 123.6, 117.7, 114.6, 60.3 ($\underline{C}HCH_2$), 55.6 (OCH_3), 42.7 ($CH\underline{C}H_2$). Calcd. for $C_{25}H_{19}N_3O_4S$: C, 65.63; H, 4.19; N, 9.18; Found: C, 65.49; H, 4.03; N, 9.32%.

*4.2.4.6. 5-{2-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-thiazolidine-2,4-dione (**4f**).* Yield 82%, mp 268–270 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 12.71 (br. s, 1H, NH), 8.27 (s, 1H, arom), 7.95–8.11 (m, 4H, arom), 7.88 (s, 1H, CH), 7.56–7.60 (m, 2H, arom), 7.40 (d, 2H, J = 8.5 Hz, arom), 7.29 (d, 2H, J = 8.5 Hz, arom), 5.71 (dd, 1H, $CH_2\underline{CH}$, J = 11.8, 4.7 Hz), 4.07 (dd, 1H, $\underline{CH_2}CH$, J = 18.2, 11.8 Hz), 3.40 (dd, 1H, $\underline{CH_2}CH$, J = 18.2, 4.7 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.2 (C=O), 167.1 (C=O), 161.2 (C=O), 157.4 (C=N), 140.7, 140.3, 134.4, 133.2, 132.7, 129.2, 129.0, 128.9, 128.8, 128.4, 128.3, 128.2, 128.1, 127.4, 123.6, 117.6, 60.3 ($\underline{C}HCH_2$), 42.6 ($CH\underline{C}H_2$). Calcd. for $C_{24}H_{16}ClN_3O_3S$: C, 62.40; H, 3.49; N, 9.10; Found: C, 62.18; H, 3.62; N, 9.02%.

*4.2.5. General procedure for synthesis of 2-{5-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethylidene]-2,4-dioxothiazolidin-3-yl}-N-arylacetamides (**5a**–**5e**)*

A suspension of compound **4b** or **4f** (3 mmol) and potassium hydroxide (3 mmol) was stirred at r.t. during 5 min, later appropriate 2-chloro-*N*-arylacetamide (3.3 mmol) was added and the mixture was refluxed for 5 h in EtOH (10 ml). Obtained powders were filtered off, washed with ethanol and recrystallized with DMF:ethanol (1:2) mixtures.

*4.2.5.1. 2-(5-{2-[5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-2,4-dioxothiazolidin-3-yl)-N-p-tolylacetamide (**5a**).* Yield 76%, mp 234–236 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 10.07 (s, 1H, NH), 8.02 (s, 1H, COCH), 7.82–7.84 (m, 2H, arom), 7.39–7.46 (m, 5H, arom), 7.31 (d, 2H, J = 8.2 Hz, arom), 7.26 (d, 2H, J = 8.2 Hz, arom), 7.04 (d, 2H, J = 7.9 Hz, arom), 5.70 (dd, 1H, $CH_2\underline{CH}$, J = 11.3, 4.2 Hz), 4.47 (s, 2H, CH_2), 3.98 (dd, 1H, $\underline{CH_2}CH$, J = 18.1, 11.3 Hz), 3.24 (dd, 1H, $\underline{CH_2}CH$, J = 18.1, 4.2 Hz), 2.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.1 (C=O), 166.1 (C=O), 163.8 (C=O), 160.9 (C=O), 157.8 (C=N), 140.5, 137.5, 136.3, 133.2, 132.7, 131.5, 130.8, 129.7, 129.4, 129.3, 128.2, 127.6, 119.7, 119.1, 60.2 ($\underline{C}HCH_2$), 44.1 (CH_2), 42.7 ($CH\underline{C}H_2$), 20.9 (CH_3) Calcd. for $C_{29}H_{23}ClN_4O_4S$: C, 62.31; H, 4.15; N, 10.02; Found: C, 62.49; H, 4.00; N, 9.89%.

*4.2.5.2. 2-(5-{2-[5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-2,4-dioxothiazolidin-3-yl)-N-(4-methoxyphenyl)-acetamide (**5b**).* Yield 72%, mp 228–230 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 10.04 (s, 1H, NH), 8.02 (s, 1H, COCH), 7.82–7.84 (m, 2H, arom), 7.43–7.52 (m, 5H, arom), 7.32 (d, 2H, J = 7.7 Hz, arom), 7.26 (d, 2H, J = 7.7 Hz, arom), 6.79 (d, 2H, J = 8.6 Hz, arom), 5.69 (dd, 1H, $CH_2\underline{CH}$, J = 11.6, 4.2 Hz), 4.44 (s, 2H, CH_2), 3.98 (dd, 1H, $\underline{CH_2}CH$, J = 17.8, 11.6 Hz), 3.75 (s, 3H, CH_3), 3.24 (dd, 1H, $\underline{CH_2}CH$, J = 17.8, 4.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.1 (C=O), 166.1 (C=O), 163.5 (C=O), 160.9 (C=O), 157.7 (C=N), 156.0, 140.5, 137.5, 132.7, 131.9, 131.5, 130.8, 129.4, 129.3, 128.3, 127.6, 121.3, 119.1, 114.4, 60.2 ($\underline{C}HCH_2$), 55.7 (OCH_3), 44.1 (CH_2), 42.7 ($CH\underline{C}H_2$). Calcd. for $C_{29}H_{23}ClN_4O_5S$: C, 60.57; H, 4.03; N, 9.74; Found: C, 60.39; H, 3.88; N, 9.58%.

*4.2.5.3. 2-(5-{2-[5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-2,4-dioxothiazolidin-3-yl)-N-(4-chlorophenyl)-acetamide (**5c**).* Yield 79%, mp 289–290 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 10.51 (s, 1H, NH), 7.98 (s, 1H, COCH), 7.83–7.86 (m, 2H, arom), 7.48–7.58 (m, 5H, arom), 7.34–7.42 (m, 3H, arom), 7.28 (d, 2H, J = 8.3 Hz, arom), 5.69 (dd, 1H, $CH_2\underline{CH}$, J = 11.2, 4.2 Hz), 4.49 (s, 2H, CH_2), 3.97 (dd, 1H, $\underline{CH_2}CH$, J = 17.5, 11.2 Hz), 3.25 (dd, 1H, $\underline{CH_2}CH$, J = 17.5, 4.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.1 (C=O), 165.1 (C=O), 164.3 (C=O), 160.9 (C=O), 157.8 (C=N), 140.5, 137.7, 137.4, 132.7, 131.5, 130.8, 129.4, 129.3, 129.2, 128.3, 127.9, 127.6, 121.3, 119.3, 60.2 ($\underline{C}HCH_2$), 44.2 (CH_2), 42.7 ($CH\underline{C}H_2$). Calcd. for $C_{28}H_{20}ClN_4O_4S$: C, 58.04; H, 3.48; N, 9.67; Found: C, 58.19; H, 3.28; N, 9.45%.

*4.2.5.4. N-(4-Methoxyphenyl)-2-(5-{2-[5-(4-methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-2,4-dioxothiazolidin-3-yl)-acetamide (**5d**).* Yield 86%, mp 268–269 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 10.24 (s, 1H, NH), 8.29 (s, 1H, COCH), 8.00–8.09 (m, 5H, arom), 7.59 (br. s, 2H, arom), 7.46 (d, 2H, J = 6.4 Hz, arom), 7.21 (d, 2H, J = 6.4 Hz, arom), 6.90 (br. s, 3H, arom), 5.68 (dd, 1H, $CH_2\underline{CH}$, J = 11.1, 4.2 Hz), 4.47 (s, 2H, CH_2), 4.05 (dd, 1H, $\underline{CH_2}CH$, J = 15.6, 11.1 Hz), 3.71 (s, 6H, 2*OCH_3), 3.42 (dd, 1H, $\underline{CH_2}CH$, J = 15.6, 4.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.4 (C=O), 167.2 (C=O), 164.3 (C=O), 160.9 (C=O), 159.2, 157.5 (C=N), 156.0, 140.1, 137.5, 134.4, 133.8, 133.2, 131.9, 129.1, 128.9, 128.7, 128.6,

128.2, 128.1, 127.6, 127.4, 123.6, 117.7, 114.6, 60.3 ($\underline{C}HCH_2$), 55.7 (OCH_3), 55.6 (OCH_3), 44.1 (CH_2), 42.7 ($CH\underline{C}H_2$). Calcd. for $C_{34}H_{28}N_4O_6S$: C, 65.79; H, 4.55; N, 9.03; Found: C, 65.96; H, 4.32; N, 8.75%.

*4.2.5.5. N-(4-Chlorophenyl)-2-(5-{2-[5-(4-methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2-oxoethylidene}-2,4-dioxothiazolidin-3-yl)-acetamide (**5e**).* Yield 87%, mp 279–280 °C. ^{1}H NMR (400 MHz, DMSO-d_6 + CCl_4): δ 10.33 (s, 1H, NH), 8.09–8.14 (m, 3H, COCH, arom), 7.87–7.93 (m, 3H, arom), 7.53–7.58 (m, 4H, arom), 7.25 (d, 2H, J = 7.4 Hz, arom), 7.19 (d, 2H, J = 6.4 Hz, arom), 6.84 (d, 2H, J = 6.4 Hz, arom), 5.68 (dd, 1H, $CH_2\underline{CH}$, J = 12.1, 3.8 Hz), 4.48 (s, 2H, CH_2), 4.03 (dd, 1H, $\underline{CH_2}CH$, J = 16.6, 12.1 Hz), 3.76 (s, 3H, OCH_3), 3.40 (dd, 1H, $\underline{CH_2}CH$, J = 16.6, 3.8 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.2 (C=O), 165.1 (C=O), 164.3 (C=O), 160.8 (C=O), 159.2, 157.9 (C=N), 137.8, 137.3, 134.4, 133.6, 133.2, 129.2, 129.1, 129.0, 128.8, 128.5, 128.2, 128.1, 127.9, 127.6, 127.4, 123.7, 121.4, 119.4, 114.7, 60.4 ($\underline{C}HCH_2$), 55.6 (OCH_3), 44.2 (CH_2), 42.8 ($CH\underline{C}H_2$). Calcd. for $C_{33}H_{25}ClN_4O_5S$: C, 63.41; H, 4.03; N, 8.96; Found: C, 63.59; H, 4.18; N, 9.09%.

4.3. Pharmacology

4.3.1. Primary anticancer assay

Primary anticancer assay was performed on a panel of approximately sixty human tumor cell lines derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda [30–33]. Tested compounds were added to the culture at a single concentration (10^{-5} M) and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). Results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The cytotoxic and/or growth inhibitory effects of the most active selected compounds were tested *in vitro* against the full panel of human tumor cell lines at concentrations ranging from 10^{-4} to 10^{-8} M. 48-h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth.

Using absorbance measurements [time zero (Tz), control growth in the absence of drug (C), and test growth in the presence of drug (Ti)], the percentage growth was calculated for each drug concentration. Percentage growth inhibition was calculated as:

$$[(Ti - Tz)/(C - Tz)] \times 100 \text{ for concentrations for which } Ti \geq Tz,$$

$$[(Ti - Tz)/Tz] \times 100 \text{ for concentrations for which } Ti < Tz.$$

Dose response parameters (GI_{50}, TGI) were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells. The drug concentration resulting in total growth inhibition (TGI) was calculated from $Ti = Tz$. Values were calculated for each of these parameters if the level of activity was reached; however, if the effect was not reached or was excessive, the value for that parameter was expressed as more or less than the maximum or minimum concentration tested. The lowest values were obtained with the most sensitive cell lines. Compounds having GI_{50} values $\leq$100 μM were declared to be active.

4.3.2. Methods for assay of antiviral activity

Primary antiviral assay was performed on a respiratory viruses panel (Flu A (H1N1), Flu A (H3N2), Flu A (H5N1), Flu B, SARS CoV) [43]. Compounds were diluted to 20 mg/ml in DMSO then eight half-log dilutions were prepared in MEM solution with 50 mg/ml gentamicin. Each dilution was added to 5 wells of a 96-well plate with 80–100% confluent cells, and three wells of each dilution were then infected with the test virus using a multiplicity of infection of <0.006 CCID50 per cell for each virus. Two wells remained uninfected as toxicity controls. A known active compound was run in parallel as a control. After cytopathic effect (CPE) was observed microscopically, plates were stained with neutral red dye for approximately 2 h, then supernatant dye was removed from the wells and the incorporated dye was extracted in 50:50 Sorensen citrate buffer/ethanol and read on a spectrophotometer at 540 nm. The optical density of test wells was converted to percent of cell and virus controls, then the concentration of test compound required to inhibit viral CPE by 50% (EC_{50}) was calculated by regression analysis. The concentration of compound that would cause 50% cytotoxicity in the uninfected cells was similarly calculated (CC_{50}). EC_{50} and CC_{50} were presented in μM. The selective index (SI) is the CC_{50} divided by EC_{50}.

4.3.3. Anti-trypanosomal activity assay

Bloodstream forms of *T. brucei brucei* strain 90-13 and *T. brucei gambiense* Feo strain were cultured in HMI9 medium supplemented with 10% FCS at 37 °C under an atmosphere of 5% CO_2 [44]. 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 [45]. Drug stock solutions were prepared in pure DMSO. *T. brucei* bloodstream forms (10^5 cells/ml) were cultured in 96-well plates either in the absence or in the presence of different concentrations of inhibitors in a final volume of 200 μl. After a 72-h incubation, resazurin solution was added in each well at the final concentration of 45 μM 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 (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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