Full Paper

Synthesis and Anticancer Activity of Isatin-Based Pyrazolines and Thiazolidines Conjugates

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The synthesis and antitumor activity screening of novel isatin based conjugates with thiazolidine and pyrazoline moieties were performed. Reaction of 3,5-diaryl-4,5-dihydropyrazoles with chloroacetyl chloride yielded starting 2-chloro-1-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-ethanones which were utilized in alkylation of isatin and 5-bromoisatin. Thus, corresponding 1-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethyl]-1*H*-indole-2,3-diones (**1a–1d**) have been obtained. The compounds **1a–1d** have been used in Knoevenagel condensation with 4-thiazolidinones for obtaining a series of 5-ylidenederivatives **2a–2f** and **3a–3d**. The synthesized compounds were tested for their anticancer activity in NCI60 cell lines. Among the tested compounds, 5-bromo-1-{2-[5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-1*H*-indole-2,3-dione (**1d**) was found to be the most active candidate with selective influence on leukemia subpanel tumor cell lines with GI_{50} values range of 0.69–3.35 μM.

Keywords: Anticancer activity / Isatines / Pyrazolines / 4-Thiazolidinones

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Introduction

The 1*H*-indole-2,3-dione [1] is the privileged scaffold in the modern medicinal chemistry which have a broad spectrum of the biological activity and the wide possibility to the chemical modification. The evaluation of antitumor activity is actual and promising for isatin derivatives [2]. The mechanisms of antitumor 1*H*-indole-2,3-diones can be associated with their affinity to tyrosine kinase [3], cyclin-dependent kinases (CDKs) [4] and carbonic anhydrase isozymes (CAIs) [5]. It is known that the combination of different bioactive fragments with complementary pharmacophoric functions or with different mechanisms of action often showed synergistic effects [6–8]. Thus, among isatin based conjugates with benzothiazole [8], thiazolidinone [9, 10] or 4-piperazinylquinoline [11] the promising anticancer agents were identified.

On the other hand diazoles (pyrazoles and pyrazolines) have occupied a unique position in the design and synthesis of novel biologically active agents that exert remarkable anticancer activities [12, 13]. The various antitumor diazole derivatives were identified as inhibitors of cyclin-dependent kinase [14], heat shock proteins [15], vascular endothelium growth factors [16], and P-glycoprotein [17]. These observations have prompted us to synthesized new isatin-pyrazoline hybrids with the hope of discovering active and selective compounds that would elicit synergistic anticancer activity. The further conjugation of isatin-pyrazoline hybrids with 4-thiazolidinones have been motivated by the significant potential of thiazolidine derivatives as antitumor agents [6]. The previous systematic study of 4-thiazolidinone derivatives with heterocyclic fragments in the molecules allowed us to identify a number of highly-active antitumor compounds *in vitro* [7, 18–21].

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The present work is an extension of our ongoing efforts towards developing promising antitumor agents by a hybride pharmacophore approach. We synthesized hybrid compounds by linking the main structural unit of the isatin ring system with the pyrazoline and 4-thiazolidinone, and examined their antitumor activity *in vitro*.

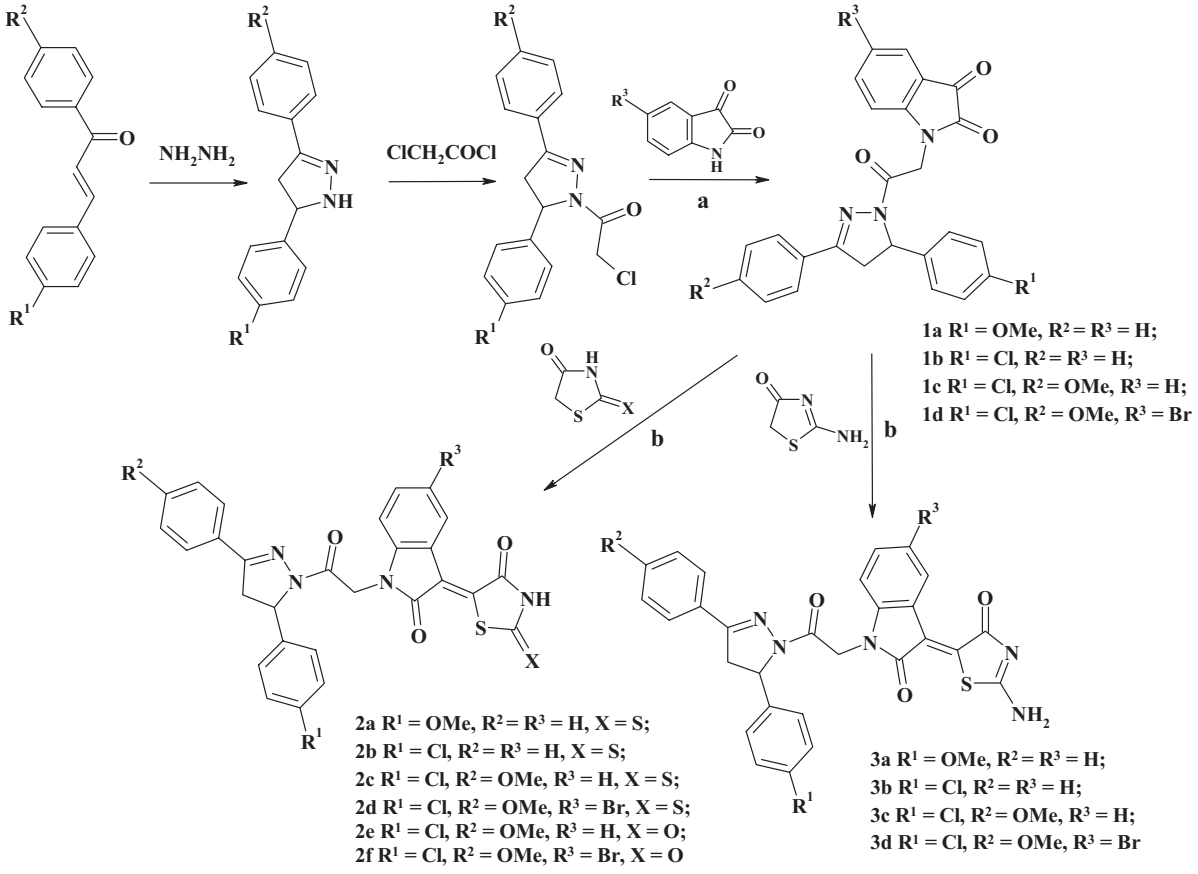
Results and discussion

Chemistry

The general methods for synthesis of target isatin based conjugates with pyrazoline and 4-thiazolidinone moieties are depicted in Scheme 1.

The starting 3,5-diaryl-4,5-dihydropyrazoles, that were synthesized using known methods from appropriate chalcones [22] easily reacted with chloroacetyl chloride yielding 2-chloro-1-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-ethanones [23]. Appropriate 2-chloro-1-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-ethanones were tested as alkylating agents in the reaction with isatin and 5-bromoisatin in DMF at room temperature [24]. Thus the corresponding 1-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethyl]-1*H*-indole-2,3-diones **1a–1d** have been obtained (Scheme 1). It is known that nature of ylidene moiety in position 5 of 4-thiazolidinone cycle has an essential influence on the antitumor activity [6, 7, 20, 25]. Relying on these observations and considering the high isatin reactivity as carbonile compound, we utilized 1-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethyl]-1*H*-indole-2,3-diones **1a–1d** in the reactions with 2,4-thiazolidinedione, 2-thioxo-4-thiazolidinone, and 2-amino-4-thiazolone according to the standard Knoevenagel condensation procedure (medium – acetic acid, catalyst – fused sodium acetate) [6, 7, 20, 25, 26]. Following the mentioned reactions the new non-condensed isatin conjugates with pyrazoline and 4-thiazolidinone **2a–2f** and **3a–3d** have been synthesized.

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



Scheme 1. Synthesis of 1,3-dihydroindol-2-one conjugates with 3,5-diaryl-4,5-dihydropyrazole and 4-thiazolidinone moieties. Reagents, conditions, and yields: (a) K_2CO_3, DMF, r.t. 12 h, 68–78%; (b) AcONa, AcOH, reflux 2–3 h, 67–79%.

Protons CH_2-CH of pyrazoline fragment in the ^{1}H-NMR spectra of synthesized compounds showed characteristic patterns of an AMX system. The chemical shifts of the protons H_A, H_M, and H_X have been assigned to about $\delta \sim 3.14–3.25$, $\delta \sim 3.87–3.95$, and $\delta \sim 5.53–5.61$, respectively with corresponding coupling constants of $J_{AM} = 16.8–18.3$, $J_{AX} = 10.8–11.8$, and $J_{MX} = 3.2–4.8$ Hz. The protons of the methylene group (CH_2CO) appear as two doublets at $\delta \sim 4.92–5.09$ ppm and $\delta \sim 5.05–5.17$ ppm. In the ^{1}H-NMR spectra of **2a–2f** NH proton of thiazolidinone cycle shows the broad singlet at $\delta \sim 8.82–9.01$ and the NH_2 protons of **3a–3d** have been found as two broad singlets and doublet at $\delta \sim 9.42–9.49$, $\delta \sim 9.19–9.26$, and $\delta \sim 9.03–9.04$ correspondingly. This could be explained by amino-imino tautomerism of these derivatives (**3a–3d**) [27].

Evaluation of anticancer activity *in vitro*

Synthesized 1-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethyl]-1*H*-indole-2,3-diones **1a**, **1b**, **1d**, and 1,3-dihydroindol-2-one conjugates with 3,5-diaryl-4,5-dihydropyrazolyne and 4-thiazolidinone moieties (**2d–2f**, **3a**, **3c**, **3d**) were submitted and evaluated at the single concentration of 10^{-5} M towards panel of approximately sixty cancer cell lines. The human tumor cell lines were derived from nine different cancer types: Leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers. Primary anticancer assays (Table 1) were performed according to the US NCI protocol, which was described elsewhere (see e.g. http://dtp.nci.nih.gov) [29–31]. The results of primary screening are reported as the percent cancer cell line growth (GP%) and presented in Table 1. The range of growth % shows the lowest and the highest growth % found among different cancer cell lines.

The most active compound **1d** was found to be effective against 26 cell lines and three compounds (**1a**, **2e**, **3a**) were found to be moderately effective against few cell lines, while five compounds (**1b**, **2d**, **2f**, **3c**, **3d**) were found to be ineffective (Table 1).

Finally, compound **1d** possessed considerable activity against most of tested human tumor cell lines and was selected in 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) (see also http://dtp.nci.nih.gov) [29–31]. Based on the cytotoxicity assays, three antitumor activity dose-response parameters were calculated for experimental agents against each cell line: GI_{50} – molar concentration of the compound that inhibits 50% net cell growth; TGI – molar concentration of the compound leading to total inhibition; and LC_{50} – molar concentration of the compound leading to 50% net cell death. Furthermore a mean graph midpoints (MG_MID) were calculated for each of the parameters, giving an average activity parameter over all cell lines for tested compounds. For the calculation of the MG_MID, insensitive cell lines were included with the highest concentration tested (Table 2).

Table 1. Anticancer screening data in concentration 10.00 μM.

Compd.	60 cell lines assay in 1 dose 10.00 μM conc.			
	Mean growth %	**Range of growth %**	**The most sensitive cell lines**	**Growth % of the most sensitive cell lines**
1a	98.37	48.40–161.24	SR (Leukemia)	48.40
			CCRF-CEM (Leukemia)	55.55
1b	104.51	74.05–171.03	SR (Leukemia)	74.05
1d	57.27	−31.51–121.19	CCRF-CEM (Leukemia)	7.75
			HL-60 (TB) (Leukemia)	5.26
			K-562 (Leukemia)	17.03
			MOLT-4 (Leukemia)	3.71
			RPMI-8226 (Leukemia)	20.92
			SR (Leukemia)	10.49
			NCI-H522 (Non-small cell lung cancer)	5.18
			HCT-116 (Colon cancer)	5.58
			KM 12 (Colon cancer)	13.70
			SW-620 (Colon cancer)	−31.51
			OVCAR-3 (Ovarian cancer)	7.45
			PC-3 (Prostate cancer)	16.98
			MCF-7 (Breast cancer)	15.31
2d	109.41	68.21–148.16	MDA-MB-231/ATCC (Breast cancer)	68.21
2e	95.41	63.86–116.92	PC-3 (Prostate cancer)	63.86
2f	101.92	74.24–134.29	SNB-75 (CNS cancer)	74.24
3a	91.84	56.94–126.98	SNB-75 (CNS cancer)	56.94
3c	104.65	84.50–150.27	SR (Leukemia)	84.50
3d	106.30	83.02–133.93	K-562 (Leukemia)	83.02

Table 2. The influence of compound **1d** on the growth of individual tumor cell lines ($GI_{50} \leq 10.00$ μM).

Disease	Cell line	GI_{50} (μM)	TGI (μM)
MG_MID		28.39	61.52
Leukemia	CCRF-CEM	0.75	11.7
Leukemia	HL-60 (TB)	2.64	11.9
Leukemia	K-562	3.35	13.5
Leukemia	MOLT-4	2.52	8.21
Leukemia	RPMI-8226	0.69	8.53
Leukemia	SR	1.69	5.71
NSC lung cancer	HOP-62	10.0	27.3
NSC lung cancer	HOP-92	3.69	17.3
NSC lung cancer	NCI-H23	8.08	>100.0
NSC lung cancer	NCI-H522	2.59	10.1
Colon cancer	HCT-116	2.00	8.73
Colon cancer	HCT-15	3.40	>100.0
Colon cancer	HT29	3.74	>100.0
Colon cancer	KM12	2.98	>100.0
Colon cancer	SW-620	2.19	5.92
CNS cancer	SF-268	8.90	>100.0
CNS cancer	SF-539	3.32	26.5
CNS cancer	SNB-75	3.05	17.5
CNS cancer	U251	3.57	35.5
Melanoma	LOX IMVI	2.70	18.8
Melanoma	SK-MEL-2	8.15	>100.0
Ovarian cancer	IGROV1	4.61	>100.0
Ovarian cancer	OVCAR-3	2.05	7.31
Ovarian cancer	OVCAR-4	2.28	96.9
Ovarian cancer	OVCAR-8	4.27	>100.0
Renal cancer	786-0	2.82	18.2
Renal cancer	CAKI-1	5.68	>100.0
Renal cancer	RXF 393	2.25	7.16
Renal cancer	TK-10	4.96	>100.0
Renal cancer	UO-31	7.77	>100.0
Prostate cancer	PC-3	2.26	>100.0
Breast cancer	MCF7	2.61	12.2
Breast cancer	HS 578T	8.46	48.6
Breast cancer	T-47D	2.32	>100.0

The tested compound **1d** showed inhibition activity against 34 ($GI_{50} < 10$ μM) from 59 human tumor cells with average GI_{50}/TGI values 28.39 μM/61.52 μM (Table 2). With regard to the sensitivity against some individual cell lines among several subpanel, compound **1d** showed the high cytostatic and cytotoxic effects against NSC lung cancer NCI-H522 cell line ($GI_{50} = 2.59$ μM, TGI = 10.1 μM), colon cancer HCT-116 ($GI_{50} = 2.00$ μM, TGI = 8.73 μM) and SW-620 ($GI_{50} = 2.19$ μM, TGI = 5.92 μM) cell lines, ovarian cancer OVCAR-3 cell line ($GI_{50} = 2.05$ μM, TGI = 7.31 μM) and renal cancer RXF 393 cell line ($GI_{50} = 2.25$ μM, TGI = 7.16 μM). It also revealed an obvious sensitivity profile of **1d** toward the all leukemia subpanel tumor cell lines with GI_{50} values range of 0.69–3.35 μM and the highest activity against CCRF-CEM ($GI_{50} = 0.75$ μM) and RPMI-8226 ($GI_{50} = 0.69$ μM) cell lines.

The selectivity index (SI) obtained by dividing the full panel MG-MID (μM) of the compound **1d** by their individual subpanel MG-MID (μM) was considered as a measure of compound's selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios greater than 6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria are rated nonselective [32]. In this context, the active compound **1d** in the present study was found to be high selectivity toward the leukemia subpanel at both the GI_{50} and TGI levels (selectivity index 14.63 and 6.19, respectively) and moderate selectivity toward the CNS cancer and renal cancer subpanels with selectivity index near 3 at the GI_{50} level (3.69 and 3.24, respectively) (Table 3).

The SAR study revealed that: (1) Hybridization of the isatin ring system with the pyrazoline ring system can lead to appearance of antitumor activity; (2) introduction of bromo group in 5-position of isatin fragment enhanced the potency of isatin-pyrazoline conjugates; (3) condensation of highly-active isatin-pyrazoline hybrid (**1d**) with 4-thiazolidinones led to complete loss of activity.

Table 3. Anticancer selectivity pattern of the most active compound **1d** at the GI_{50} (μM) and TGI (μM) levels.

Disease	GI_{50}	SI[a]	TGI	SI[b]
Leukemia	1.9	14.63	9.9	6.19
NSC lung cancer	45.4	0.68	72.7	0.85
Colon cancer	61.6	0.46	84.7	0.73
CNS cancer	7.7	3.69	53.6	1.15
Melanoma	56.5	0.50	81.8	0.75
Ovarian cancer	17.2	1.65	78.7	0.78
Renal cancer	8.8	3.24	72.5	0.84
Prostate cancer	31.8	0.89	>100.0	0.62
Breast cancer	24.5	1.16	72.3	0.85

[a] Selectivity index at the GI_{50} level. [b] Selectivity index at the TGI level.

COMPARE analysis

NCI's COMPARE algorithm (see e.g. http://dtp.nci.nih.gov) [33, 34] allows to assume biochemical mechanisms of action of novel compounds on the basis of their *in-vitro* activity profiles when comparing with those of standard agents. We performed COMPARE computations for synthesized highly-active compound **1d** against the NCI "Standard Agents" database at the GI_{50} and TGI levels (Table 4). However obtained Pearson correlation coefficients (PCC) preclude that the compound may have the same mechanism of action as that of known anticancer agents. The compound **1d** showed the moderate correlations at the TGI level with microtubulin formation-modulating agents (microtubule polymerization inhibitors) – macrolides rhizoxin and maytansine (Table 4). The isatin-pyrazoline conjugates do not belong to a class of these antitumor antibiotics, however, according to the literature data, the tubulin polymerization inhibitors were identified among isatin [2, 35–37] and pyrazoline [13] derivatives. This fact prompts us to further study of the antitumor activity mechanism of isatin-pyrazoline conjugates.

Conclusions

In the present paper new isatin based conjugates with pyrazoline and 4-thiazolidinone moieties were described. Antitumor activity testing allowed us to identify highly active isatin-pyrazoline hybrid **1d**, as prospective anticancer agent with high selective influence on the leukemia subpanel at both the GI_{50} and TGI levels (selectivity index 14.63 and 6.19, respectively) and moderate selectivity toward the CNS cancer and renal cancer subpanels with selectivity index near 3 at the GI_{50} level. The highest activity compound **1d** demonstrated on the leukemia subpanel tumor cell lines with GI_{50} values range of 0.69–3.35 μM. The further investigations of such isatin derivatives could be interesting with the hope to get more selective anticancer agents among isatin-pyrazoline hydrid analoges.

Experimental

Materials and methods

The starting 2-chloro-1-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-ethanones [23], 2,4-thiazolidinedione [38], 2-amino-4-thiazolone [38], 2-thioxo-4-thiazolidinone [39] were obtained according to the methods described previously.

Melting points were measured in open capillary tubes on a BÜCHI 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 300 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.

Chemistry

*Synthesis of 1-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethyl]-1H-indole-2,3-diones (**1a–1d**)*

A mixture of 50 mmol isatin or 5-bromoisatin, 60 mmol of appropriate 2-chloro-1-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-etha-

Table 4. COMPARE analysis results for highly-active compound **1d**.

End point	PCC[a]	Target	Target vector NSC	Target mechanism of action[b]
GI_{50}	0.509	Rifamycin	S133100	DNA-polymerase inhibitor
TGI	0.581	Rhizoxin	S332598	Microtubulin formation-modulating agent (microtubule polymerization inhibitor)
	0.561	Maytansine	S153858	Microtubulin formation-modulating agent (microtubule polymerization inhibitor)
	0.533	L-Cysteine analogue	S303861	Reversible binding inhibitor of the human kinesin Eg5, antimitotic agent
	0.529	CCNU	S79037	Chloroalkylating agent
	0.524	Methyl-CCNU	S95441	Chloroethylating alkylator, alkyl transferase-dependent cross-linkers
	0.505	Macbecin II	S269148	Heat shock protein (Hsp-90) inhibitor

[a] Only correlations with PCC ≥ 0.5 were selected, as significant. [b] Putative mechanisms of action were identified with the use of literature sources.

none, and 100 mmol of potassium carbonate was stirred in 30 mL of DMF at room temperature during 12 h. The crystalline products were separated by filtration, washed with water, ethanol, and dried. Recrystallization from mixture DMF/ethanol rendered desired products in pure form.

*1-{2-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-1H-indole-2,3-dione (**1a**)*

Yield 68%, mp 236–238°C. IR [cm^{-1}]: 1736 (CO), 1664 (CO), 1616 (CO), 1448 (arom), 756. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 7.87–7.83 (m, 2H, arom); 7.65–7.48 (m, 5H, arom); 7.16–7.09 (m, 4H, arom); 6.87 (d, 2H, J = 8.7 Hz, arom); 5.55 (dd, 1H, J = 11.6, 4.6 Hz, CH); 5.11 (d, 1H, J = 17.5 Hz, CH_2); 4.96 (d, 1H, J = 17.5 Hz, CH_2); 3.89 (dd, 1H, J = 18.3, 11.6 Hz, CH_2CH); 3.69 (s, 3H, CH_3); 3.15 (dd, 1H, J = 18.3, 4.6 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 163.9 (C=O), 161.8 (C=O), 158.46 (C=O), 151.8 (C=N), 141.0, 138.8, 131.3, 129.2, 129.0, 128.0, 127.4, 124.8, 123.6, 114.6, 110.2, 60.2 (*C*HCH$_2$), 55.5 (OCH_3), 42.8 (CH*C*H$_2$), 42.3 (CH_2). Calcd. for $C_{26}H_{21}N_3O_4$: C, 71.06; H, 4.82; N, 9.56; Found: C, 71.20; H, 4.70; N, 9.75%.

*1-{2-[5-(4-Chlorophenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-1H-indole-2,3-dione (**1b**)*

Yield 75%, mp 256–258°C. IR [cm^{-1}]: 1742 (CO), 1658 (CO), 1632 (CO), 1456 (arom), 748. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 7.86–7.84 (m, 2H, arom); 7.62 (t, 1H, J = 7.8 Hz, arom); 7.58 (d, 1H, J = 7.4 Hz, arom); 7.54–7.50 (m, 3H, arom); 7.37 (d, 2H, J = 8.4 Hz, arom); 7.29 (d, 2H, J = 8.4 Hz, arom); 7.15 (t, 1H, J = 7.4 Hz, arom); 7.11 (d, 1H, J = 7.9 Hz, arom); 5.60 (dd, 1H, J = 11.8, 4.8 Hz, CH); 5.05 (d, 1H, J = 17.4 Hz, CH_2); 4.94 (d, 1H, J = 17.4 Hz, CH_2); 3.89 (dd, 1H, J = 18.3, 11.8 Hz, CH_2CH); 3.25 (dd, 1H, J = 18.3, 4.8 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 164.3 (C=O), 163.8 (C=O), 158.6 (C=O), 156.4 (C=N), 140.9, 138.9, 138.8, 131.2, 129.2, 129.0, 128.1, 127.4, 124.9, 123.8, 117.6, 111.7, 60.1 (*C*HCH$_2$), 42.6 (CH*C*H$_2$), 42.3 (CH_2). Calcd. for $C_{25}H_{18}ClN_3O_3$: C, 67.64; H, 4.07; N, 9.47; Found: C, 67.52; H, 3.98; N, 9.55%.

*1-{2-[5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-1H-indole-2,3-dione (**1c**)*

Yield 71%, mp 245–246°C. IR [cm^{-1}]: 1725 (CO), 1658 (CO), 1632 (CO), 1462 (arom), 752. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 7.81 (d, 2H, J = 8.6 Hz, arom); 7.63 (t, 1H, J = 7.4 Hz, arom); 7.58 (d, 1H, J = 7.0 Hz, arom); 7.39 (d, 2H, J = 8.2 Hz, arom); 7.27 (d, 2H, J = 8.2 Hz, arom); 7.14 (d, 2H, J = 6.0 Hz, arom); 7.06 (d, 2H, J = 8.6 Hz, arom); 5.57 (dd, 1H, J = 11.8, 3.2 Hz, CH); 5.05 (d, 1H, J = 17.6 Hz, CH_2); 4.92 (d, 1H, J = 17.6 Hz, CH_2); 3.89 (dd, 1H, J = 18.0, 11.8 Hz, CH_2CH); 3.83 (s, 3H, CH_3); 3.22 (dd, 1H, J = 18.0, 3.2 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 163.5 (C=O), 161.8 (C=O), 158.6 (C=O), 151.6 (C=N), 141.1, 132.4, 129.4, 128.9, 128.0, 124.8, 123.8, 123.5, 114.7, 111.7, 59.9 (*C*HCH$_2$), 55.8 (OCH_3), 42.5 (CH*C*H$_2$), 42.3 (CH_2). Calcd. for $C_{26}H_{20}ClN_3O_4$: C, 65.90; H, 4.25; N, 8.87; Found: C, 65.79; H, 4.18; N, 8.95%.

*5-Bromo-1-{2-[5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-1H-indole-2,3-dione (**1d**)*

Yield 78%, mp 258–260°C. IR [cm^{-1}]: 1740 (CO), 1668 (CO), 1608 (CO), 1444 (arom), 1256, 1176, 832. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 7.81–7.77 (m, 3H, arom); 7.72 (s, 1H, arom); 7.37 (d, 1H, J = 8.0 Hz, arom); 7.28 (d, 2H, J = 8.0 Hz, arom); 7.11 (d, 1H, J = 8.3 Hz, arom); 7.05 (d, 2H, J = 8.4 Hz, arom); 5.58 (dd, 1H, J = 11.8, 4.0 Hz, CH); 5.08 (d, 1H, J = 17.5 Hz, CH_2); 4.96 (d, 1H, J = 17.5 Hz, CH_2); 3.93 (dd, 1H, J = 18.1, 11.8 Hz, CH_2CH); 3.84 (s, 3H, CH_3); 3.22 (dd, 1H, J = 18.1, 4.0 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 163.5 (C=O), 162.8 (C=O), 158.8 (C=O), 152.1 (C=N), 142.2, 136.6, 135.2, 133.8, 129.4, 129.0, 128.1, 123.8, 123.5, 114.7, 111.7, 59.9 (*C*HCH$_2$), 55.8 (OCH_3), 42.5 (CH*C*H$_2$), 42.3 (CH_2). Calcd. for $C_{26}H_{19}BrClN_3O_4$: C, 56.49; H, 3.46; N, 7.60; Found: C, 56.58; H, 3.38; N, 7.75%.

*General procedure for synthesis of 5-(1-{2-[3,5-diaryl-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-4-thiazolidinones (**2**) and 2-amino-4-thiazolones (**3**)*

A mixtures of compound **1a–1d** (3.3 mmol), appropriate 4-thiazolidinone derivative (3 mmol), and anhydrous sodium acetate (3 mmol) were refluxed for 2 h in glacial acetic acid (10 mL). Obtained powders were filtered off, washed with methanol, and recrystallized with DMF/ethanol (1:2) mixture.

*5-(1-{2-[5-(4-Methoxyphenyl)-3-phenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-2-thioxo-4-thiazolidinone (**2a**)*

Yield 71%, mp 290–292°C. IR [cm^{-1}]: 1740 (CO), 1680 (CO), 1612 (CO), 1520 (C=C), 1440 (arom), 1232, 1184, 1088 (C=S), 760. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 8.84 (br.s, 1H, NH); 7.85 (d, 2H, J = 6.9 Hz, arom); 7.50–7.48 (m, 3H, arom); 7.42 (t, 1H, J = 7.6 Hz, arom); 7.16–7.13 (m, 3H, arom); 7.04 (d, 1H, J = 7.7 Hz, arom); 6.88 (d, 2H, J = 6.9 Hz, arom); 5.53 (dd, 1H, J = 11.4, 4.6 Hz, CH); 5.14 (d, 1H, J = 17.2 Hz, CH_2); 5.02 (d, 1H, J = 17.2 Hz, CH_2); 3.90 (dd, 1H, J = 18.0, 11.4 Hz, CH_2CH); 3.74 (s, 3H, CH_3); 3.15 (dd, 1H, J = 18.0, 4.6 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.8 (C=S), 168.9 (C=O), 163.3 (C=O), 161.8 (C=O), 151.8 (C=N), 141.0, 138.7, 132.3, 129.2, 129.0, 128.0, 124.8, 123.6, 123.5, 114.7, 110.7, 60.1 (*C*HCH$_2$), 55.7 (OCH_3), 42.7 (CH*C*H$_2$), 42.3 (CH_2). Calcd. for $C_{29}H_{22}N_4O_4S_2$: C, 62.80; H, 4.00; N, 10.10; Found: C, 62.87; H, 3.38; N, 10.19%.

*5-(1-{2-[5-(4-Chlorophenyl)-3-phenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-2-thioxo-4-thiazolidinone (**2b**)*

Yield 67%, mp 270–272°C. IR [cm^{-1}]: 1740 (CO), 1680 (CO), 1608 (CO), 1536 (C=C), 1440 (arom), 1232, 1180, 1088 (C=S), 752. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 8.85 (br.s, 1H, NH); 7.82 (br.s, 2H, arom); 7.63 (t, 1H, J = 7.6 Hz, arom); 7.50 (br.s, 3H, arom); 7.43 (t, 1H, J = 7.7 Hz, arom); 7.37 (d, 2H, J = 8.4 Hz, arom); 7.29 (d, 2H, J = 8.4 Hz, arom); 7.15 (t, 1H, J = 7.3 Hz, arom); 7.09 (d, 1H, J = 7.7 Hz, arom); 5.61 (dd, 1H, J = 11.4, 4.8 Hz, CH); 5.17 (d, 1H, J = 17.3 Hz, CH_2); 5.09 (d, 1H, J = 17.3 Hz, CH_2); 3.95 (dd, 1H, J = 17.8, 11.4 Hz, CH_2CH); 3.15 (dd, 1H, J = 17.8, 4.8 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.6 (C=S), 168.8 (C=O), 163.8 (C=O), 160.8 (C=O), 154.6 (C=N), 140.8, 138.9, 138.8, 131.3, 129.2, 129.0, 128.2, 127.8, 124.8, 123.5, 117.7, 111.5, 60.1 (*C*HCH$_2$), 42.6 (CH*C*H$_2$), 42.3 (CH_2). Calcd. for $C_{28}H_{19}ClN_4O_3S_2$: C, 60.16; H, 3.43; N, 10.02; Found: C, 60.29; H, 3.35; N, 10.14%.

*5-(1-{2-[5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-2-thioxo-4-thiazolidinone (**2c**)*

Yield 75%, mp 288–289°C. IR [cm^{-1}]: 1745 (CO), 1682 (CO), 1615 (CO), 1542 (C=C), 1445 (arom), 1180, 1088 (C=S), 752. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 8.82 (br.s, 1H, NH); 7.79 (d, 2H, J = 6.1 Hz, arom); 7.45 (t, 1H, J = 7.2 Hz, arom); 7.37 (d, 2H, J = 7.8 Hz, arom); 7.25 (d, 2H, J = 7.8 Hz, arom); 7.15 (t, 1H, J = 7.3 Hz, arom); 7.07–7.03 (m, 3H, arom); 5.57 (dd, 1H, J = 11.1, 4.2 Hz, CH); 5.15 (d, 1H, J = 17.3 Hz, CH_2); 5.03 (d, 1H, J = 17.3 Hz, CH_2); 3.92 (dd, 1H, J = 17.8, 11.1 Hz, CH_2CH); 3.83 (s, 3H, CH_3); 3.17 (dd, 1H, J = 17.8, 4.2 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.8 (C=S), 168.6 (C=O), 163.7 (C=O), 161.0 (C=O), 152.6 (C=N), 141.2, 138.9, 132.4, 131.3, 129.4, 129.0, 128.0, 127.9, 124.8, 123.7, 114.8, 111.7, 60.0 (*C*HCH$_2$), 55.8 (OCH$_3$), 42.6 (CH*C*H$_2$), 42.3 (CH$_2$). Calcd. for $C_{29}H_{21}ClN_4O_4S_2$: C, 59.13; H, 3.59; N, 9.51; Found: C, 59.02; H, 3.45; N, 9.34%.

*5-(5-Bromo-1-{2-[5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-2-thioxo-4-thiazolidinone (**2d**)*

Yield 70%, mp 284–286°C. IR [cm^{-1}]: 1740 (CO), 1672 (CO), 1608 (CO), 1520 (C=C), 1432 (arom), 1232, 1176, 1088 (C=S), 832. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.01 (br.s, 1H, NH); 7.81–7.77 (m, 3H, arom); 7.60 (s, 1H, arom); 7.37 (d, 2H, J = 7.6 Hz, arom); 7.25 (d, 2H, J = 7.6 Hz, arom); 7.06–7.02 (m, 3H, arom); 5.54 (dd, 1H, J = 11.2, 3.4 Hz, CH); 5.15 (d, 1H, J = 17.1 Hz, CH_2); 5.02 (d, 1H, J = 17.1 Hz, CH_2); 3.87 (dd, 1H, J = 17.8, 11.2 Hz, CH_2CH); 3.83 (s, 3H, CH_3); 3.19 (dd, 1H, J = 17.8, 3.4 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 188.6 (C=S), 168.2 (C=O), 163.5 (C=O), 162.0 (C=O), 152.2 (C=N), 142.0, 136.9, 135.2, 133.7, 131.4, 129.2, 128.2, 127.9, 124.7, 123.8, 114.8, 111.8, 59.9 (*C*HCH$_2$), 55.7 (OCH$_3$), 42.5 (CH*C*H$_2$), 42.3 (CH$_2$). Calcd. for $C_{29}H_{20}BrClN_4O_4S_2$: C, 52.14; H, 3.02; N, 8.39; Found: C, 52.22; H, 2.95; N, 8.31%.

*5-(1-{2-[5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-2,4-thiazolidinedione (**2e**)*

Yield 69%, mp 248–250°C. IR [cm^{-1}]: 1736 (CO), 1705 (CO), 1672 (CO), 1612 (CO), 1536 (C=C), 1448 (arom), 1256, 1176, 832, 752. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 8.83 (br.s, 1H, NH); 7.79 (d, 2H, J = 8.4 Hz, arom); 7.63 (t, 1H, J = 7.4 Hz, arom); 7.58 (d, 1H, J = 7.8 Hz, arom); 7.38 (d, 2H, J = 7.6 Hz, arom); 7.27 (d, 2H, J = 7.6 Hz, arom); 7.15–7.09 (m, 2H, arom); 7.05 (d, 2H, J = 8.4 Hz, arom); 5.58 (dd, 1H, J = 11.1, 3.2 Hz, CH); 5.08 (d, 1H, J = 17.6 Hz, CH_2); 4.95 (d, 1H, J = 17.6 Hz, CH_2); 3.93 (dd, 1H, J = 17.7, 11.1 Hz, CH_2CH); 3.84 (s, 3H, CH_3); 3.17 (dd, 1H, J = 17.7, 3.2 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 163.5 (C=O), 161.7 (C=O), 158.7 (C=O), 156.2 (C=O), 151.5 (C=N), 141.0, 138.8, 132.3, 129.1, 129.0, 128.0, 124.8, 123.8, 123.5, 114.7, 111.7, 59.9 (*C*HCH$_2$), 55.8 (OCH$_3$), 42.5 (CH*C*H$_2$), 42.3 (CH$_2$). Calcd. for $C_{29}H_{21}ClN_4O_5S$: C, 60.79; H, 3.69; N, 9.78; Found: C, 60.87; H, 3.55; N, 9.64%.

*5-(5-Bromo-1-{2-[5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-2,4-thiazolidinedione (**2f**)*

Yield 75%, mp 280–282°C. IR [cm^{-1}]: 1744 (CO), 1704 (CO), 1676 (CO), 1608 (CO), 1520 (C=C), 1460 (arom), 1312, 1256, 1180, 824. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 8.99 (br.s, 1H, NH); 7.77–7.71 (m, 3H, arom); 7.58 (br.s, 1H, arom); 7.35 (d, 2H, J = 7.6 Hz, arom); 7.26 (d, 2H, J = 7.6 Hz, arom); 7.09–7.04 (m, 3H, arom); 5.57 (dd, 1H, J = 10.8, 4.7 Hz, CH); 5.15 (d, 1H, J = 17.8 Hz, CH_2); 5.03 (d, 1H, J = 17.8 Hz, CH_2); 3.92 (dd, 1H, J = 16.8, 10.8 Hz, CH_2CH); 3.84 (s, 3H, CH_3); 3.20 (dd, 1H, J = 16.8, 4.7 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 164.1 (C=O), 162.6 (C=O), 159.5 (C=O), 157.2 (C=O), 152.3 (C=N), 142.4, 136.4, 135.4, 132.4, 129.2, 129.1, 128.1, 128.0, 123.7, 114.7, 59.9 (*C*HCH$_2$), 55.8 (OCH$_3$), 42.3 (CH*C*H$_2$), 42.2 (CH$_2$). Calcd. for $C_{29}H_{20}BrClN_4O_5S$: C, 53.43; H, 3.09; N, 8.59; Found: C, 53.32; H, 3.15; N, 8.47%.

*5-(1-{2-[5-(4-Methoxyphenyl)-3-phenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-2-amino-4-thiazolone (**3a**)*

Yield 72%, mp 276–278°C. IR [cm^{-1}]: 2860, 1700 (CO), 1676 (CO), 1612 (CO), 1538 (C=C), 1440 (arom), 1360, 1180. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.42, 9.21, 9.03 (br.s, s, d, 2H, NH_2); 7.85 (d, 2H, J = 7.7 Hz, arom); 7.50–7.48 (m, 3H, arom); 7.35 (t, 1H, J = 7.6 Hz, arom); 7.16 (d, 2H, J = 8.5 Hz, arom); 7.12 (t, 1H, J = 7.6 Hz, arom); 6.99 (d, 1H, J = 7.7 Hz, arom); 6.88 (d, 2H, J = 8.5 Hz, arom); 5.53 (dd, 1H, J = 11.4, 4.3 Hz, CH); 5.15 (d, 1H, J = 17.3 Hz, CH_2); 5.03 (d, 1H, J = 17.3 Hz, CH_2); 3.93 (dd, 1H, J = 18.1, 11.4 Hz, CH_2CH); 3.74 (s, 3H, CH_3); 3.22 (dd, 1H, J = 18.1, 4.3 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 163.9 (C=O), 158.9 (C=O), 156.2 (C=O), 152.4 (C=N), 143.8, 134.1, 131.5, 131.2, 131.0, 129.2, 128.0, 127.3, 122.7, 120.2, 114.5, 109.6, 60.2 (*C*HCH$_2$), 55.5 (OCH$_3$), 42.9 (CH*C*H$_2$), 42.3 (CH$_2$). Calcd. for $C_{29}H_{23}N_5O_4S$: C, 64.79; H, 4.31; N, 13.03; Found: C, 64.88; H, 4.39; N, 13.19%.

*5-(1-{2-[5-(4-Chlorophenyl)-3-phenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-2-amino-4-thiazolone (**3b**)*

Yield 69%, mp 280–282°C. IR [cm^{-1}]: 2850, 1688 (CO), 1670 (CO), 1608 (CO), 1536 (C=C), 1440 (arom), 1362, 1180. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.19, 9.04 (br.s, d, 2H, NH_2); 7.83 (d, 2H, J = 7.7 Hz, arom); 7.59–7.47 (m, 3H, arom); 7.34 (br.s, 3H, arom); 7.27 (d, 2H, J = 7.7 Hz, arom); 7.17 (t, 1H, J = 7.5 Hz, arom); 6.98 (d, 1H, J = 7.2 Hz, arom); 5.61 (dd, 1H, J = 11.3, 4.5 Hz, CH); 5.15 (d, 1H, J = 17.2 Hz, CH_2); 5.03 (d, 1H, J = 17.2 Hz, CH_2); 3.93 (dd, 1H, J = 18.1, 11.3 Hz, CH_2CH); 3.22 (dd, 1H, J = 18.1, 4.3 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 163.7 (C=O), 159.2 (C=O), 158.8 (C=O), 151.8 (C=N), 143.0, 138.9, 138.0, 131.7, 131.2, 131.0, 129.4, 128.0, 127.3, 124.7, 123.2, 117.5, 109.6, 60.0 (*C*HCH$_2$), 42.7 (CH*C*H$_2$), 42.3 (CH$_2$). Calcd. for $C_{28}H_{20}ClN_5O_3S$: C, 62.05; H, 3.72; N, 12.92; Found: C, 62.19; H, 3.58; N, 12.74%.

*5-(1-{2-[5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-2-amino-4-thiazolone (**3c**)*

Yield 79%, mp 269–271°C. IR [cm^{-1}]: 2860, 1694 (CO), 1672 (CO), 1608 (CO), 1538 (C=C), 1434 (arom), 1356, 1180. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.49, 9.26, 9.03 (br.s, s, d, 2H, NH_2); 7.96 (d, 2H, J = 8.4 Hz, arom); 7.65 (t, 1H, J = 7.4 Hz, arom); 7.59 (d, 1H, J = 7.4 Hz, arom); 7.38 (d, 2H, J = 7.6 Hz, arom); 7.25 (d, 2H, J = 7.6 Hz, arom); 7.12–7.08 (m, 2H, arom); 7.03 (d, 2H, J = 8.4 Hz, arom); 5.56 (dd, 1H, J = 11.2, 4.3 Hz, CH); 5.14 (d, 1H,

J = 17.3 Hz, CH_2); 5.02 (d, 1H, J = 17.3 Hz, CH_2); 3.90 (dd, 1H, J = 18.2, 11.2 Hz, CH_2CH); 3.83 (s, 3H, CH_3); 3.14 (dd, 1H, J = 18.2, 4.3 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 163.8 (C=O), 159.9 (C=O), 158.6 (C=O), 151.6 (C=N), 143.2, 141.0, 138.2, 132.7, 131.2, 131.0, 129.4, 128.0, 124.8, 123.8, 123.5, 115.0, 110.9, 60.0 ($CHCH_2$), 55.8 (OCH_3), 42.6 ($CHCH_2$), 42.3 (CH_2). Calcd. for $C_{29}H_{22}ClN_5O_4S$: C, 60.89; H, 3.88; N, 12.24; Found: C, 61.02; H, 3.75; N, 12.36%.

*5-(5-Bromo-1-{2-[5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-2-oxoethyl}-2-oxo-1,2-dihydroindol-3-ylidene)-2-amino-4-thiazolone (**3d**)*

Yield 70%, mp 275–276°C. IR [cm^{-1}]: 2856, 1696 (CO), 1672 (CO), 1608 (CO), 1536 (C=C), 1432 (arom), 1360, 1180. ^{1}H-NMR (300 MHz, DMSO-d_6 + CCl_4): δ 9.44, 9.23 (br.s, s, 2H, NH_2); 7.97 (s, 1H, arom); 7.76 (d, 2H, J = 8.4 Hz, arom); 7.52 (d, 1H, J = 8.3 Hz, arom); 7.35 (d, 2H, J = 8.2 Hz, arom); 7.25 (d, 2H, J = 8.2 Hz, arom); 7.04 (d, 2H, J = 8.4 Hz, arom); 6.98 (d, 1H, J = 8.3 Hz, arom); 5.57 (dd, 1H, J = 11.4, 4.6 Hz, CH); 5.14 (d, 1H, J = 17.2 Hz, CH_2); 5.01 (d, 1H, J = 17.2 Hz, CH_2); 3.91 (dd, 1H, J = 18.1, 11.4 Hz, CH_2CH); 3.84 (s, 3H, CH_3); 3.22 (dd, 1H, J = 18.1, 4.6 Hz, CH_2CH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 164.1 (C=O), 159.2 (C=O), 158.8 (C=O), 151.8 (C=N), 142.2, 136.8, 135.2, 132.7, 131.2, 129.4, 128.1, 127.8, 123.8, 114.8, 60.0 ($CHCH_2$), 55.8 (OCH_3), 42.7 ($CHCH_2$), 42.3 (CH_2). Calcd. for $C_{29}H_{21}BrClN_5O_4S$: C, 53.51; H, 3.25; N, 10.76; Found: C, 53.38; H, 3.37; N, 10.69%.

Pharmacology

Primary anticancer assay was performed at approximately sixty human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda (see e.g. http://dtp.nci.nih.gov) [29–31]. 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 about 60 human tumor cell lines at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. A 48-h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth.

Using the seven absorbance measurements (time zero, (T_z), control growth in the absence of drug, (C), and test growth in the presence of drug at the five concentration levels (T_i)), the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$$[(T_i - T_z)/(C - T_z)] \times 100 \text{ with } T_i \geq T_z$$
$$[(T_i - T_z)/T_z] \times 100 \text{ with } T_i < T_z$$

Three dose response parameters (GI_{50}, TGI, LC_{50}) were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from:

$$[(T_i - T_z)/(C - T_z)] \times 100 = 50$$

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 $T_i = T_z$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from:

$$[(T_i - T_z)/T_z] \times 100 = -50$$

Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less than the maximum or minimum concentration tested. The lowest values are obtained with the most sensitive cell lines. Compounds having $GI_{50} \leq 100$ μM were declared to be active.

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