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RESEARCH ARTICLE

Some pharmacological properties of 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one

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Abstract: A series of 3,5-diaryl pyrazolyl thiazolinones were designed and synthesized as potential biologically active compounds. The study of anticancer activity of 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) revealed its high antiproliferative activity against a panel of cancer cells with the lowest growth inhibition concentration (GI_{50}) towards leukemic cell line SR (0.0351 µM) and ovarian cancer cell line OVCAR-3 (0.248 µM). It was also found that pyrazolyl thiazolinone **1** inhibited growth of *Trypanosoma brucei brucei* by 98,8% at a concentration of 10 µg/mL. The in-depth cytotoxicity study of compound **1** on human hepatocellular carcinoma HepG2 cells and non-tumorigenic murine fibroblast Balb/c 3T3 in MTT, NRU, TPC and LDH assays showed that normal cells were less sensitive to compound **1** than the cancer cells; its action had led to a disintegration of the cell membrane, inhibition of mitochondrial and lysosomal activity, and proliferation of cancer cells. The highest selectivity were detected in the LDH assay.

Keywords: pyrazolyl thiazolinone hybrids; antitumor activity; antitrypanosomal activity; cytotoxicity.

Introduction

The hybrid pharmacophore approach has been used to develop a drug-like molecules with anticancer properties [1]. Pyrazole and thiazole/thiazolidinone cycles are considered as favored scaffolds and have been used to design novel drug-like molecules possessing antiproliferative activity [2-3]. A combination of the above heterocycles in one molecule is likely to provide more efficient pyrazolyl-thiazole/thiazolidone conjugates via synergistic anticancer effects. For example, a 2-amino-iminothiazolidinone derivative with a pyrazole moiety at position 5 inhibited necroptosis [4], pyrazolyl-4-thiazolidinones exhibited dose-dependent cytotoxic effect in human breast cancer cells (MCF-7 line) [5], a series of 2-(5-aryl-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-1,3-thiazol-4(5*H*)ones inhibited growth of leukemia cell lines and non-small cell lung cancer cell lines [6]. Cytotoxicity of pyrazole derivatives includes inhibition of the topoisomerase I and II activity for pyrazoloacridine [7] or inhibition of the Janus-activated kinase (JAK1/2) for the Ruxolitinib [8]. The pyrazolyl thiazol/thiazolidinone-based hybrids as biologically active compounds have been studied for anti-parasitic activity [9-10]. For example, some of the 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones showed IC_{50} level of activity within 0.6-0.7 µM *in vitro* assay towards *Trypanosoma brucei gambiense* [11]; 5-[5-aryl-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-thiazolidine-2,4-dione and 2-{5-[5-aryl-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-2,4-dioxothiazolidin-3-yl}-N-arylacetamides inhibited growth of the *Trypanosoma brucei brucei* and *Trypanosoma brucei gambiense* at micromolar concentrations [12]. Thiazole/thiazolidinone phenylindole/imidazothiadiazoles comprise another highly active class of antitrypanosomals [13]. The combination of different pharmacophores in one molecule may lead to novel drug-like molecules that will exhibit biological activity. Thus, the above mentioned polypharmacological [14] and hybrid-pharmacophore approaches [1] inspire the design and synthesis of pyrazolyl-thiazolinones as potential anticancer and antiparasitic agents.

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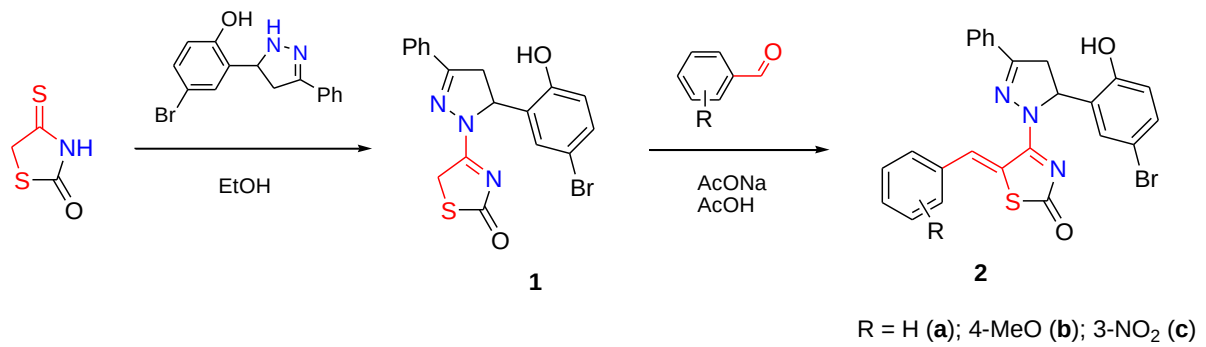

Scheme 1. Synthesis of a pyrazolyl-thiazolinone **1** and its 5-arylidene derivatives **2a-c**.

Results and Discussion

A 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) was synthesized in the reaction of 3,5-diarylpyrazoline with 4-thioxo-2-thiazolidinone (isorhodanine) in the ethanol by known approach [6]. 5-arylidene derivatives **2a-c** were synthesized in high yields by the Knoevenagel reaction with corresponding aldehydes (Scheme 1). 4-[3-(5-Bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydro-pyrazol-2-yl]-5*H*-thiazol-2-one (**1**) was selected by National Cancer Institute (NCI, Bethesda, USA) Developmental Therapeutic Program (DTP) for anticancer screening at one dose assay (10^{-5} M) using a panel of 59 cancer cell lines that represented different types of cancer (leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers). The anticancer activity of compound **1** showed the mean growth percent for the whole cancer cell panel and it was about 25% [15-17]. Moreover, the tested pyrazolyl thiazolinone **1** demonstrated the cytotoxic effects to five leukemic cell lines (CCRF-CEM, HL-60(TB), MOLT-4, RPMI-8226, SR), a non-small cell lung cancer cell line (HOP-92), a CNS cancer cell line (SF-295), and an ovarian cancer cell line (IGROV1). The high growth inhibition rates of compound **1** justified the in-depth screening at a range of concentrations using 55 cell lines. The percentage of growth was evaluated spectrophotometrically versus control that were not treated with tested compound after 48 h of exposure. The SRB protein assay was used to estimate cells' viability or growth. Three dose-response parameters for antitumor activity were found for each cell line: GI_{50} – molar concentration of the compound that inhibits 50% net cell growth; TGI – molar concentration of the compound leading to the total inhibition; and LC_{50} – molar concentration of the compound leading to 50% net cell death. The high antiproliferative activity of compound **1** against the majority of cell is reported in Table 1. The lowest inhibitory concentrations for compound **1** were observed against the leukemia panel. The most sensitive leukemic cell line was the SR line (GI_{50} = 0.0351 μM). It inhibited growth of CCRF-CEM, HL-60, MOLT-4 and SR leukemia lines at submicromolar concentrations (TGI). The high cytotoxic activity of **1** was found against non-small cell lung cancer line NCI-H322M and EKVX as well as colon cancer line HCT-116, ovarian cancer OVCAR-3, and breast cancer MCF7 lines.

The antitrypanosomal activity of pyrazolyl-thiazolinone **1** on against *T. brucei brucei* was investigated *in vivo* by the polypharmacological approach. A combination of pyrazoline and thiazolinone cycles in one molecule did not only contribute to the anticancer properties, but also increased the antiparasitic activity. It was found that 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihyd-ropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) inhibited growth of *T. brucei brucei* by 98,8% at a concentration of 10 μg/mL. Lowering concentration to 1 μg/mL led to sharp decrease in the inhibition activity (11,87 % of inhibition).

The biological assays demonstrated high antitumor and antitrypanosomal activities of pyrazolyl thiazolinone **1**. High activities inspired further in-depth studies of **1** against human hepatocellular carcinoma HepG2 cells and non-tumorigenic murine fibroblast Balb/c 3T3. Four different biochemical endpoints were measured: mitochondrial activity, lysosomal activity, total protein content, and cellular membrane integrity in MTT assay, NRU assay, TPC assay and LDH assay respectively (after 24, 48 and 72 h exposition).

The cells viability after the exposure to tested compound was found to be a time-, a concentration- and a cell line-dependent (Figure 1). Human hepatoma HepG2 cells were more sensitive to the compound **1** compared to normal murine Balb/c 3T3 fibroblast cells. The first sign of anticancer activity of compound **1** was seen at the lowest concentrations after 24 h of exposure (LDH assay). Moreover, the toxic effect for normal fibroblasts cell line in the same assay was observed at a concentration of 0.9 μM (CC_{20}) after 48 h of exposure (Figure 1). The calculated cytotoxic concentrations values (CC_{20}, CC_{50}, and CC_{80}) for the cancer HepG2 cell line and for the non-tumorigenic immortalized Balb/c 3T3 cell line are shown in Table 2. It is worth to note that the lowest cytotoxic concentrations (CC_{20}) that inhibits 20% of cell growth were calculated for all tests regardless of the exposure time. CC_{50} values indicated that the first step of anticancer action of compound **1** was the first disintegration of cellular membranes leading to the inhibition of mitochondrial activity with further inhibition of proliferation and lysosomal activity. It is noteworthy that compound **1** exhibits low toxicity against normal murine embryonic fibroblast cell line (Balb/c 3T3). Its toxic action led to dis-

Table 1. Influence of the 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) on the growth of the most sensitive tumor cell lines in *in vitro* test (10^{-4}-10^{-8}M).

Cancer cell lines		GI_{50}, μM	TGI, μM	LC_{50}, μM
Leukemia	*CCRF-CEM*	0.339	2.70	>100.0
	HL-60	0.306	0.923	>100.0
	K-562	0.959	>100.0	>100.0
	MOLT-4	0.397	17.7	>100.0
	SR	0.0351	0.282	>100.0
Non-small cell lung cancer	*A549/ATCC*	6.55	>100.0	>100.0
	EKVX	0.382	>100.0	>100.0
	NCI-H23	0.819	>100.0	>100.0
	NCI-H322M	0.306	15.3	>100.0
	NCI-H460	0.619	>100.0	>100.0
Colon cancer	*HCC-2998*	1.94	8.58	>100.0
	HCT-116	0.302	>100.0	>100.0
	HCT-15	0.767	>100.0	>100.0
	SW-620	0.694	>100.0	>100.0
CNS cancer	*SF-268*	0.805	>100.0	>100.0
	SF-295	0.636	>100.0	>100.0
Melanoma	*MALME-3M*	0.843	22.3	>100.0
	M14	0.907	28.4	>100.0
	MDA-MB-435	1.92	>100.0	>100.0
	SK-MEL-5	1.18	20.8	>100.0
Ovarian cancer	*OVCAR-3*	0.248	0.682	>100.0
	OVCAR-4	0.464	>100.0	>100.0
	NCI/ADR-RES	0.830	20.7	>100.0
	SK-OV-3	1.84	36.0	>100.0
Renal cancer	*ACHN*	1.44	>100.0	>100.0
	CAKI-1	0.723	4.06	>100.0
	TK-10	1.62	>100.0	>100.0
Prostate cancer	*DU-145*	2.24	>100.0	>100.0
Breast cancer	*MCF7*	0.301	-	>100.0
	T-47D	0.377	>100.0	>100.0
	MDA-MB-468	0.631	67.6	>100.0

integration of the cell membrane, inhibition of mitochondrial and lysosomal activity of cancer cells.

The selectivity indexes (SI), which indicates the cytotoxic selectivity (i.e. drug safety) were calculated for HepG2 cell line against corresponding normal cell line (Figure 2). Higher values of SI indicate higher anticancer specificity. Compounds with SI above 3.0 are considered highly selective [18]. The highest values of SI were detected in the LDH assay for compound **1**. The SI increased from 167 to 4975 with increase of the exposure time from 24 h to 72 h whereas SI values for the cisplatin increased only from 13 to 1429 (Figure 2).

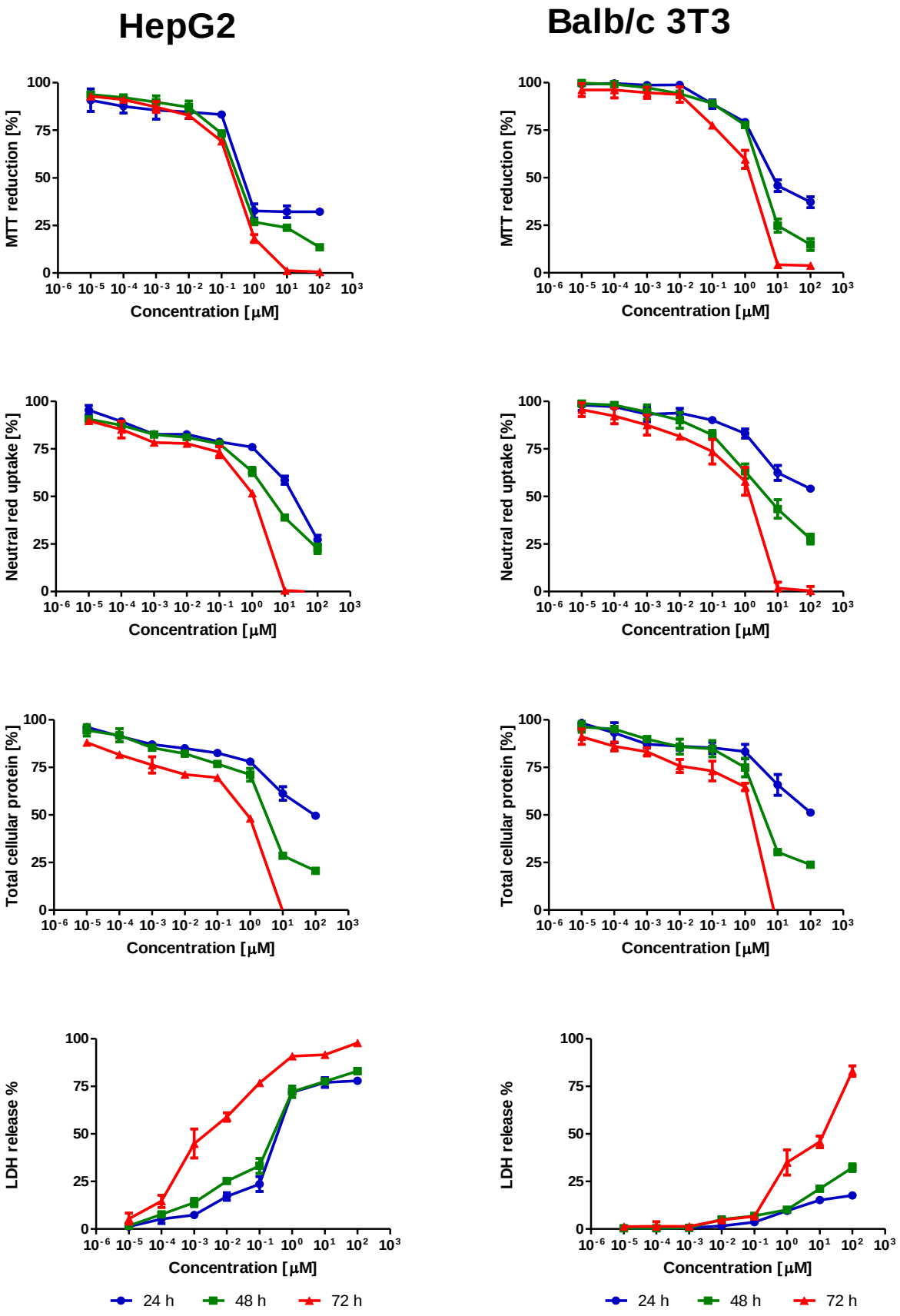

Figure 1. Cytotoxic concentrations (CC_{20}; CC_{50}; CC_{80}) (μM) calculated for compound **1** in normal and cancer cells.

Conclusions

High anticancer activity of 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) against a series of cancer cell lines with increased selectivity against leukemic cell lines had been demonstrated. (3,4-Dihydropyrazol-2-yl)-5*H*-thiazol-2-one (**1**) showed a good antitrypanosomal activity in the *in vitro* experiment against *T. brucei brucei*. The dual anticancer and antitrypanosomal activity along with high selectivity indices as well as low cytotoxicity would justify further in-depth studies of compound **1** as a perpective drug candidate.

Table 2. The values of cytotoxic concentrations (CC_{20}; CC_{50}; CC_{80}) (μM) calculated for compound **1** in MTT, NRU, TPC and LDH assays.

Cell line	Method	Time (h)	CC_{20}	CC_{50}	CC_{80}*
Balb/c 3T3	MTT	24	0.9±0.3	8.9±0.9	
		48	0.8±0.2	5.8±0.6	52.1±5.2
		72	0.1±0.1	2.5±0.8	7.4±0.5
	NRU	24	2.4±1.1	-	-
		48	0.2±0.1	7.1±1.5	
		72	0.05±0.002	2.1±1.0	7.1±0.3
	TPC	24	2.3±1.0	-	-
		48	0.6±0.3	6.0±0.5	
		72	0.006±0.002	2.8±0.7	6.5±0.9
	LDH	24	-	-	-
		48	9.2±0.6	-	-
		72	0.5±0.1	19.9±2.6	92.9±2.5
HepG2	MTT	24	0.9±0.09	6.9±0.5	-
		48	0.1±0.04	5.5±0.2	42.1±2.5
		72	0.03±0.01	0.44±0.02	0.97±0.04
	NRU	24	0.07±0.02	34.2±1.7	-
		48	0.04±0.01	5.9±0.7	-
		72	0.001±0.0003	1.3±0.1	6.6±0.1
	TPC	24	0.7±0.2	95.5±0.5	-
		48	0.05±0.02	5.4±0.4	-
		72	0.001±0.0002	0.9±0.04	6.2±0.1
	LDH	24	0.06±0.01	0.6±0.02	-
		48	0.006±0.001	0.5±0.07	47.9±4.9
		72	0.0003±0.0001	0.004±0.001	0.3±0.07

* CC_{20}, CC_{50} and CC_{80} (μM) represents the concentrations of compound **1** that is required for 20%, 50% and 80 % inhibition using the MTT, NRU, TPC, and LDH assays. Data are expressed as the mean ± SEM and were calculated from the dose response curves of at least three independent experiments.

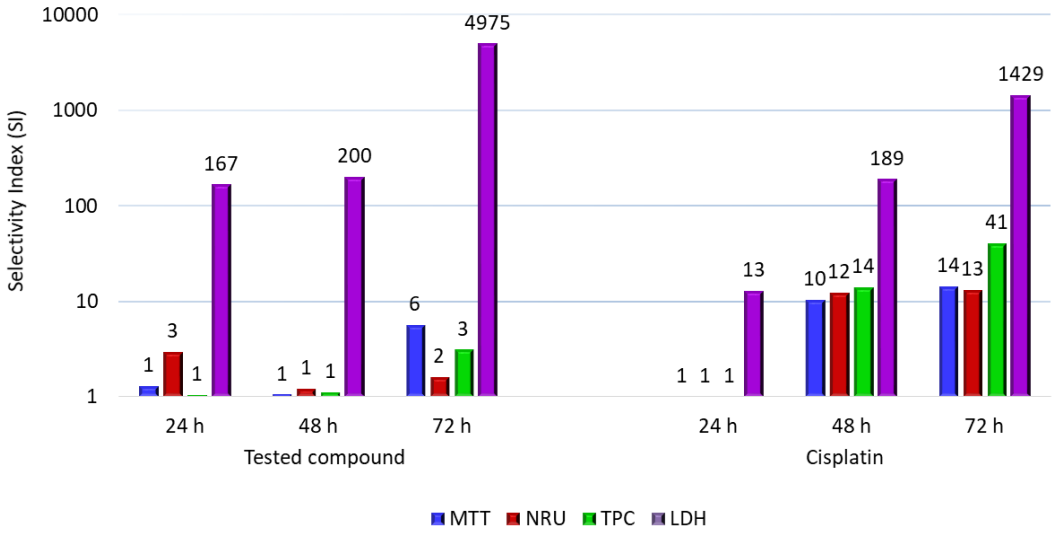

Figure 2. Selectivity indices (SI) calculated for compound **1** and cisplatin against HepG2 cells.

Experimental section

Chemistry

Commercial reagents were purchased from Merck and used without purification. Melting points were measured in open capillary tubes on a BŰCHI B-545 melting point apparatus and are uncorrected. The elemental analyses were performed using the Perkin-Elmer 2400 CHN analyzer. The ^{1}H NMR spectra were recorded on Varian Gemini (^{1}H at 400 and ^{13}C at 100 MHz) instrument in DMSO-d_6 using tetramethylsilane (TMS) as an internal standard.

The starting 3,5-diaryl-4,5-dihydropyrazole was synthesized according to known method from chalcone [19].

*4-[3-(5-Bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5H-thiazol-2-one (**1**).*

A mixture of 4-thioxo-2-thiazolidinone (0.01 mol) and 4-bromo-2-(3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol (0.01 mol) was refluxed in 100 ml of ethanol for 1.5 h. After cooling the reaction mixture to room temperature, formed precipitate was filtered off, washed with methanol and recrystallized. Yield: 65%; pale yellow solid; mp 232-234 °C, (DMF-EtOH 1:2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* 8.2 Hz, 2H, Ar), 7.47-7.56 (m, 3H, Ar), 7.24 (dd, *J* 2.7, 8.2 Hz, 1H, Ar), 7.10 (d, *J* 2.7 Hz, 1H, Ar), 6.83 (d, *J* 8.2 Hz, 1H, Ar), 5.75 (dd, *J* 3.3, 20.0 Hz, 1H, C_5H-pyraz.), 4.87 (d, *J* 16.8 Hz, 1H, C_4H-thiaz.), 4.65 (d, *J* 16.8 Hz, 1H, C_4H-thiaz.), 4.00 (dd, *J* 11.1, 20.0 Hz, 1H, C_4H-pyraz.), 3.25 (dd, *J* 3.3, 20.0 Hz, 1H, C_4H-pyraz.). Anal. Calcd. for $C_{18}H_{14}BrN_3O_2S$: C, 51.93; H, 3.39; N, 10.09. Found: C, 52.05; H, 3.45; N, 9.93.

*General procedure for synthesis of compounds **2a-c***

The equimolar amounts of 4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5*H*-thiazol-2-one (**1**) (0.01 mol) with the appropriate aromatic aldehyde (0.01 mol) and sodium acetate (0.01 mol) in the acetic acid medium were refluxed for 3-4 hours. After cooling the reaction mixture formed precipitate was filtered off and recrystallized.

*5-Benzylidene-4-[3-(5-bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]thiazol-2-one (**2a**).*

Yield: 72%; yellow solid; mp 242-244 °C, (DMF-EtOH 1:3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, 2H, *J* 8.2 Hz, Ar), 7.66 (m, 4H, Ar), 7.40-7.55 (m, 5H, Ar, C*H*=), 7.19 (d, 1H, Ar), 7.04 (d, *J* 7.3 Hz, 1H, Ar), 6.84 (d, *J* 8.0 Hz, 1H, Ar), 6.04 (dd, *J* 3.3, 20.0 Hz, 1H, C_5H-pyraz.), 3.98 (dd, *J* 11.1, 20.0 Hz, 1H, C_4H-pyraz.), 3.26 (dd, *J* 3.3, 20.0 Hz, 1H, C_4H-pyraz.). Anal. Calcd. for $C_{25}H_{18}BrN_3O_2S$: C, 59.53; H, 3.60; N, 8.33. Found: C, 59.62; H, 3.68; N, 8.29.

*4-[3-(5-Bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5-[(4-methoxyphenyl)methylene]thiazol-2-one (**2b**).*

Yield: 78%; yellow-orange solid; mp > 250 °C, (DMF-EtOH 1:3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H, O*H*), 7.88-7.93 (m, 2H, Ar), 7.64 (d, *J* 8.0 Hz, 2H, Ar), 7.50-7.54 (m, 3H, Ar, C*H*=), 7.19 (d, 1H, Ar), 7.06 (d, *J* 7.3 Hz, 3H, Ar), 6.83 (d, *J* 8.0 Hz, 1H, Ar), 6.03 (dd, *J* 3.3, 20.0 Hz, 1H, C_5H-pyraz.), 3.98 (dd, *J* 11.1, 20.0 Hz, 1H, C_4H-pyraz.), 3.29 (dd, *J* 5.0, 10.0 Hz, 1H, C_4H-pyraz.), 1.91 (s, 3H, CH_3). Anal. Calcd. for $C_{28}H_{20}BrN_3O_3S$: C, 58.43; H, 3.77; N, 7.86. Found: C, 58.50; H, 3.85; N, 7.80.

*4-[3-(5-Bromo-2-hydroxyphenyl)-5-phenyl-3,4-dihydropyrazol-2-yl]-5-[(3-nitrophenyl)methylene]thiazol-2-one (**2c**).*

Yield: 69%; yellow-orange solid; mp > 250 °C, (DMF-EtOH 1:3). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.26 (s, 1H, O*H*), 9.16 (s, 1H, Ar), 8.36 (d, 2H, *J* 8.0 Hz, Ar), 7.98-8.10 (m, 4H, Ar), 7.60 (m, 3H, Ar, C*H*=), 7.31 (d, 1H, Ar), 7.20 (s, 1H, Ar), 6.86 (d, *J* 8.0 Hz, 1H, Ar), 6.02 (dd, *J* 3.3, 20.0 Hz, 1H, C_5H-pyraz.), 4.09 (dd, *J* 11.1, 20.0 Hz, 1H, C_4H-pyraz.), 3.39 (dd, *J* 5.0, 10.0 Hz, 1H, C_4H-pyraz.). Anal.

Calcd. for $C_{25}H_{19}BrN_4O_4S$: C, 54.45; H, 3.47; N, 10.16. Found: C, 54.60; H, 3.40; N, 10.03.

Biological tests

Anticancer in vitro screening methodology as well as data interpretation are described in details at the NCI Development Therapeutics Program site [20].

Cytotoxicity assays

The human hepatoma cell line (HepG2) was purchased from the American Type Culture Collection (ATCC HB-8065). The cells were cultured in Minimum Essential Medium Eagle (MEME) (ATCC, USA). The murine fibroblasts cell line (Balb/c 3T3 clone A31) (gift from Department of Swine Diseases of the National Veterinary Research Institute in Pulawy, Poland) was cultured in Dulbecco`s Modified Eagle`s Medium (DMEM) (ATCC, USA). The media were supplemented with 10% BCS (Balb/c 3T3), 10% FBS (HepG2), 1% L-glutamine, 1% antibiotic solution. The cells were maintained in 75 cm^2 cell culture flasks (NUNC) in a humidified incubator at 37 °C, in an atmosphere of 5% CO_2. The medium was refreshed every two or three days and the cells were trypsinized by 0.25% trypsin-0.02% EDTA after reaching 70-80% confluence. Single cell suspensions were prepared and adjusted to a density of $2x10^5$ cell/mL (HepG2) and $1x10^5$ cell/mL for 24h, 48h exposition or $5x10^4$ cell/mL for 72 h exposition (Balb/c 3T3). The cell suspension was transferred to 96-well plates (100 µl/well) and incubated for 24 h before the exposure to the studied compound. Stock solution of the studied compound was prepared in DMSO and diluted with culture medium to obtain a concentration range from 10^{-5}-10^2 µM. The cells were also exposed to the reference drug – cisplatin used as internal laboratory control. The final concentration of DMSO was 0.1% in the medium and had no influence on cell growth. The medium used for test solutions and in control preparation did not contain serum and antibiotics. As negative control, cultured cells were grown in the absence of study compound. Each concentration was tested in six replicates with three independent experiments. Cytotoxicity was assessed after 24, 48 and 72 h of exposure the cells to tested compound. The medium was not changed during the incubation time.

MTT assay. The metabolic activity of living cells was assessed by the measurement of the activity of dehydrogenases [21].

NRU assay. The method is based on staining living cells with neutral red which readily diffuses through the plasma membrane and accumulates in lysosomes [22].

TCP assay. The assay was based upon staining total cellular protein (proliferation) [23].

LDH leakage assay. The integrity of the plasma membrane was assessed through the test of lactate dehydrogenase (LDH) release [24], which was monitored using the commercially available Cytotoxicity Detection Kit (LDH) (Roche Diagnostics, Poland). The absorbance was measured at microplate reader (Synergy HTXmulti-mode reader (BioTek® Instruments Inc., USA)) at 570 nm, using blank as a references. Cytotoxicity was expressed as a percentage of the negative control (0.1% DMSO) [25].

Selectivity index.

To determine the cytotoxic selectivity of the tested compound, the selectivity index (SI) was calculated according to the following equation:

$$SI = CC_{50}^{\text{no cancer cells}}/CC_{50}^{\text{cancer cells}}$$

If a SI was found to be ≥ 3 the compound can be considered to be selective [18].

A beneficial SI > 3.0 indicates a drug with efficacy against tumor cells greater than toxicity against normal cells. The results of the cytotoxicity assessment were expressed as mean arithmetic values from three independent experiments. The percentage of viability inhibition was calculated in comparison with the untreated controls. The CC_{20}, CC_{50}, CC_{80} values (cytotoxicity concentrations) are the compound's concentrations that inhibit the cell viability by 20%, 50% and 80%were calculated by GraphPad Prism 5 software (San Diego, CA, USA) using nonlinear regression.

Notes

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Деякі фармакологічні властивості 4-[3-(5-бромо-2-гідроксифеніл)-5-феніл-3,4-дигідропіразол-2-іл]-5Н-тіазол-2-ону

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Резюме: Розроблено та синтезовано ряд піразоліл-тіазолідинонів як потенційних біологічно активних сполук. Дослідження протипухлинної активності 4-[3-(5-бром-2-гідроксифеніл)-5-феніл-3,4-дигідропіразол-2-іл]-5*H*-тіазол-2-ону (**1**) виявило його високі антипроліферативні властивості щодо панелі більш, ніж 50-ти ракових клітинних ліній з найнижчою концентрацією пригнічення росту (GI_{50}) обчисленою для лінії лейкемії SR (0,0351 мкМ) та лінії раку яйників OVCAR-3 (0,248 мкМ). Крім того, піразоліл-тіазолідинон **1** повністю пригнічував ріст (TGI) ліній лейкемії CCRF-CEM, HL-60, MOLT-4 та SR при субмікромолярних значеннях концентрації. Крім ліній лейкемії, чутливими до дії сполуки **1** були лінії недрібноклітинного раку легень *NCI-H322M* та *EKVX*, лінія раку товстого кишківника *HCT-116*, раку яйників *OVCAR-3* та раку молочної залози MCF7. Також було встановлено, що піразоліл-тіазолідинон **1** інгібує ріст *Trypanosoma brucei brucei* на 98,8% у концентрації 10 мкг/мл, що свідчить про перспективність розробки даного агенту в рамках концепції «поліфармакологічних лікарських засобів». Поглиблене дослідження цитотоксичності сполуки **1** на клітинах гепатоцелюлярної карциноми людини HepG2 та нормальних мишачих фібробластах Balb/c3T3 у MTT, NRU, TPC та LDH тестах показало, що нормальні клітини менш чутливі до дії піразоліл-тіазолідинону **1**, ніж ракові. Відповідно до значень CC_{50}, першим етапом протипухлинної дії сполуки **1** було пошкодження клітинних мембран, що призводить до інгібування мітохондріальної активності, з послідовним інгібуванням проліферації та лізосомальної активності ракових клітин. Для 4-[3-(5-бром-2-гідроксифеніл)-5-феніл-3,4-дигідропіразол-2-іл]-5*H*-тіазол-2-ону (**1**) обчислено індекси селективності, що були вищими у тесті LDH, ніж такі для препарату порівняння цисплатину.

Ключові слова: піразолін-тіазолідинонові гібриди; протипухлинна активність; антитрипаносомна активність; цитотоксичність.