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Synthesis and evaluation of biological activity of rhodanine-pyrazoline hybrid molecules with a 2-(2,6-dichlorophenylamino)-phenylacetamide fragment

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Aim. Synthesis of new rhodanine-pyrazoline hybrid molecules with a diclofenac fragment in position 3 using the reactions of heterocyclization and aminolysis with potential antitumor and antitrypanosomal activities. **Methods.** Organic synthesis, NMR spectroscopy, pharmacological screening. **Results.** The reaction between 2-(2,6-dichloro-phenyl-amino)-phenylacetic acid hydrazide and thiocarbonyl-bis-thioglycolic acid in ethanol medium providing rhodanine derivative with fragment of the anti-inflammatory drug diclofenac in position 3 was performed. The presence of an active methylene group in position 5 and its subsequent modification providing 5-ethoxymethylenerhodanine and further aminolysis reaction with various 3,5-diaryl-4,5-dihydro-1*H*-pyrazoles allowed to produce of a series of 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones. The antitumor activity screening allowed to identify a highly active compound 9 with a mean GI_{50} = 0.71/1.09 and TGI = 82.95/28.46 μM towards 60 cancer lines (DTP NCI program). The synthesized pyrazoline-thiazolidine hybrid molecules with the diclofenac fragment in the structure did not show significant antitrypanosomal activity against *Trypanosoma brucei brucei (Tbb)*. **Conclusions.** The synthesized 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones with a diclofenac fragment in the structure are a promising molecular platform for creation of new highly active potential drugs with a low toxicity.

Keywords: synthesis, 2-thioxo-4-thiazolidinone, diclofenac, spectral data, antitumor activity, an-titrypanosomal activity

Introduction

The rhodanine heterocyclic framework represents privileged structural motif of many compounds displaying interesting activities against a diverse set of biological targets [1,2]. Thus, among 2-thioxothiazolidin-4-one (rhodanine) structural-type compounds the set of potent anticancer [3], antifungal [4], antiviral [5], antimicrobial [6], antimalarial [7] and anti-inflammatory [8, 9] agents has been identified. The synthesis and biological activity evaluation of rhodanine-related molecules are based on the previous results of experimental studies of these heterocycle derivatives as potential chemotherapeutic agents [10-12]. Antitumor activity evaluation associated with the affinity to diverse biotargets is promising for these type compounds. Thus, among rhodanine derivatives it has been identified several proto-oncogene serine/threonine protein kinase (Pim-1,2,3) [13], protein disulfide isomerase [14], c-Myc-Max [15], Bcl-2 and Bax family [16], JNK-stimulating phosphatase-1 (JSP-1) inhibitors [17]. Additionally, rhodanine fragment is the basis of potential antitrypanosomal agents, especially among 5-benzylidenerhodanine-3-acetic acid derivatives with inhibitory activity on dolicholphosphate mannose synthase (DPMS) and glycosylphosphatidylinositol (GPI) anchor synthesis against *T. brucei* bloodstream forms *in vitro* [18]. Among rhodanine-based conjugates with pyrazoline moiety at 5 position the highest active trypanocidal agents against *T. brucei* gambiense (Feo strain) has been identified with IC_{50} values in the range of 0.6-0.7 μM [19]. Accordingly, in our further research we accomplished the synthesis of novel hybrid molecules, which combines the biologically active scaffolds of pyrazole, rhodanine as well as the fragment of non-steroidal anti-inflammatory drug (NSAID) diclofenac. Noteworthy, diclofenac-based molecules along with the main anti-inflammatory effect possess a wide spectrum of other biological activities, in particular, anticancer [20] and antioxidant [21]. Thus, the purpose of our work was the design and synthesis of 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones with a diclofenac moiety, and evaluation of their trypanocidal and antitumor activities.

Materials and methods

Chemistry

All materials were purchased from Merck and Sigma-Aldrich and were used without purification. Melting points were measured in open capillary tubes and are uncorrected. Elemental analyses were performed using Perkin-Elmer 2400 CHN analyzer. The ^{1}H NMR (400 MHz) spectra were recorded on Varian Mercury 400 (400 MHz) in DMSO-d_6 using tetramethylsilane as internal standard. Mass spectra were obtained using electrospray ionization (ESI) techniques on an Agilent 1100 Series LC/MSD. The purity of all obtained compounds was checked by thin-layer chromatography (eluent benzene : EtOAc 1:1).

Synthesis of 2-[2-(2,6-dichlorophenylamino)-phenyl]-N-(5-ethoxymethylene-4-oxo-2-thioxothiazolidin-3-yl)-acetamide (**2**). A mixture of 2-[2-(2,6-dichloro-phenylamino)-phenyl]-*N*-(4-oxo-2-thioxothiazolidin-3-yl)-acetamide (10 mmol) and triethyl orthoformate

was heated at reflux for 2 h in acetic anhydride (10 mL). The resulting solution was poured into water, extracted with ethyl acetate, after which the organic layer was distilled off in vacuo. Obtained powder was recrystallized from acetic acid. Yield 67 %, mp 234-235ºC. ^{1}H NMR: δ 1.33 (t, 3H, J = 7.1 Hz, CH_3), 2.38 (m, 2H, CH_2), 4.17 (s, 2H, CH_2), 6.28 (s, 1H, =CH), 6.65 (t, 1H, J = 7.6 Hz, arom.), 6.86 (t, 1H, J = 7.6 Hz, arom.), 7.07 (t, 1H, J = 8.1 Hz, arom.), 7.07 (d, 1H, J = 7.6 Hz, arom.), 7.19 (d, 1H, J = 7.6 Hz, arom.), 7.52 (d, 2H, J = 8.1 Hz, arom.), 8.29 (s, 1H, NH), 11.50 (s, 1H, NH). Anal.Calcd for $C_{20}H_{17}Cl_2N_3O_3S_2$: C, 49.80; H, 3.55; N, 8.71. Found: C, 49.72; H, 3.39; N, 8.62. ESI-MS m/z 481.4/483.4 $(M+H)^+$.

General procedure for the synthesis of 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones (**3-11**). A mixture of compound **2** (10 mmol) with the appropriate 3,5-diaryl-4,5-dihydro-1*H*-pyrazole (10 mmol) was refluxed for 2 h in the ethanol medium. The obtained solid products were filtered off, washed with ethanol and recrystallized from the mixture of DMF – EtOH (1:1).

N-{5-[3,5-Bis-(4-chlorophenyl)-4,5-dihydropyrazol-1-ylmethylene]-4-oxo-2-thioxothiazolidin-3-yl}-2-[2-(2,6-dichlorophenylamino)-phenyl]-acetamide (***3***). Yield 71 %, mp 203-204ºC. ^{1}H NMR: δ 3.47 (dd, 1H, J = 18.0, 6.4 Hz, CH_2), 3.74 (s, 2H, CH_2), 3.99 (dd, 1H, J = 18.0, 11.2 Hz, CH_2), 5.63 (dd, 1H, J = 11.2, 6.4 Hz, CH), 6.99-7.04 (m, 3H, arom.), 7.14 (d, 2H, J = 8.4 Hz, arom.), 7.17 (d, 2H, J = 8.6 Hz, arom.), 7.21 (d, 2H, J = 8.4 Hz, arom.), 7.23 (d, 2H, J = 8.6 Hz, arom.), 7.26-7.34 (m, 4H, arom.), 7.99 (s, 1H, =CH), 10.58 (s, 1H, NH), 11.39 (s, 1H, NH). Anal.Calcd for $C_{33}H_{23}Cl_4N_5O_2S_2$: C, 54.48; H, 3.19; N, 9.63. Found: C, 54.55; H, 3.10; N, 9.52. ESI-MS m/z 726.5/728.5 $(M+H)^+$.

N-{5-[3-(4-Chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazol-1-ylmethylene]-4-oxo-2-thioxothiazolidin-3-yl}-2-[2-(2,6-dichlorophenylamino)-phenyl]-acetamide (**4**). Yield 78 %, mp 193-194ºC. ^{1}H NMR: δ 3.45 (dd, 1H, J = 18.3, 6.8 Hz, CH_2), 3.71 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.97 (dd, 1H, J = 18.3, 11.0 Hz, CH_2), 5.60 (dd, 1H, J = 11.0, 6.8 Hz, CH), 6.83 (t, 1H, J = 7.0 Hz, arom.), 6.94-7.02 (m, 3H, arom.), 7.14 (t, 1H, J = 8.2 Hz, arom.), 7.26-7.35 (m, 3H, arom.), 7.40 (m, 1H, arom.),7.45 (dd, 2H, J = 3.5, 8.0 Hz, arom.), 7.63 (d, 2H, J = 8.4 Hz, arom.), 7.83-7.89 (m, 2H, arom.), 7.94 (s, 1H, =CH), 10.57 (s, 1H, NH), 11.31 (s, 1H, NH). Anal. Calcd for $C_{34}H_{26}Cl_3N_5O_3S_2$: C, 56.48; H, 3.62; N, 9.69. Found: C, 56.57; H, 3.70; N, 9.62. ESI-MS m/z 722.1/724.1 $(M+H)^+$.

N-{5-[5-(4-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydropyrazol-1-ylmethylene]-4-oxo-2-thioxothiazolidin-3-yl}-2-[2-(2,6-dichlorophenylamino)-phenyl]-acetamide (**5**). Yield 65 %, mp 188-189ºC. ^{1}H NMR: δ 3.51 (dd, 1H, J = 18.6, 5.9 Hz, CH_2), 3.85 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 4.07 (dd, 1H, J = 18.6, 10.8 Hz, CH_2), 5.74 (dd, 1H, J = 10.8, 5.9 Hz, CH), 7.07 (t, 1H, J = 7.0 Hz, arom.),7.16-7.21 (m, 4H, arom.), 7.43 (d, 2H, J = 8.6 Hz, arom.), 7.50-7.52 (m, 5H, arom.), 7.77 (s, 1H, =CH), 7.83 (d, 1H, J = 8.8 Hz, arom.), 7.86 (d, 2H, J = 8.6 Hz, arom.), 10.61 (s, 1H, NH), 11.35 (s, 1H, NH). Anal.Calcd

for $C_{34}H_{26}Cl_3N_5O_3S_2$: C, 56.48; H, 3.62; N, 9.69. Found: C, 56.52; H, 3.69; N, 9.77. ESI-MS m/z 722.1/724.1 $(M+H)^+$.

N-{5-[3,5-Bis-(4-methoxyphenyl)-4,5-dihydropyrazol-1-ylmethylene]-4-oxo-2-thioxothiazolidin-3-yl}-2-[2-(2,6-dichlorophenylamino)-phenyl]-acetamide (**6**). Yield 81 %, mp 166-167ºC. ^{1}H NMR: δ 3.39 (dd, 1H, *J* = 18.0, 6.5 Hz, CH_2), 3.71 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 4.05 (dd, 1H, *J* = 18.0, 11.4 Hz, CH_2), 5.50 (dd, 1H, *J* = 11.4, 6.5 Hz, CH), 7.00-7.05 (m, 3H, arom.), 7.13 (d, 2H, *J* = 8.4 Hz, arom.), 7.19 (d, 2H, *J* = 8.6 Hz, arom.), 7.21 (d, 2H, *J* = 8.4 Hz, arom.), 7.23 (d, 2H, *J* = 8.6 Hz, arom.), 7.21-7.33 (m, 4H, arom.), 8.04 (s, 1H, =CH), 10.59 (s, 1H, NH), 11.31 (s, 1H, NH). Anal.Calcd for $C_{35}H_{29}Cl_2N_5O_4S_2$: C, 58.49; H, 4.07; N, 9.74. Found: C, 58.62; H, 4.15; N, 9.62. ESI-MS m/z 717.6/719.6 $(M+H)^+$.

2-[2-(2,6-Dichlorophenylamino)-phenyl]-N-{5-[5-(4-methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-4-oxo-2-thioxothiazolidin-3-yl}-acetamide (**7**). Yield 71 %, mp 190-191ºC. ^{1}H NMR: δ 3.51 (dd, 1H, *J* = 18.8, 6.8 Hz, CH_2), 3.76 (s, 2H, CH_2), 3.77 (s, 3H, OCH_3), 4.04 (dd, 1H, *J* = 18.8, 10.7 Hz, CH_2), 5.64 (dd, 1H, *J* = 10.7, 6.8 Hz, CH), 6.88 (t, 1H, *J* = 8.2 Hz, arom.), 6.99-7.02 (m, 2H, arom.), 7.06 (t, 1H, *J* = 7.6 Hz, arom.), 7.18 (t, 1H, *J* = 8.0 Hz, arom.), 7.31-7.39 (m, 4H, arom.), 7.45 (m, 1H, arom.), 7.50-7.53 (m, 2H, arom.), 7.58-7.61 (m, 4H, arom.), 7.68 (s, 1H, =CH), 11.35 (s, 1H, NH), 11.37 (s, 1H, NH). Anal.Calcd for $C_{34}H_{27}Cl_2N_5O_3S_2$: C, 59.30; H, 3.95; N, 10.17. Found: C, 59.42; H, 3.88; N, 10.22. ESI-MS m/z 687.6/689.6 $(M+H)^+$.

2-[2-(2,6-Dichlorophenylamino)-phenyl]-N-{5-[5-(4-dimethylaminophenyl)-3-phenyl-4,5-dihydropyrazol-1-ylmethylene]-4-oxo-2-thioxothiazolidin-3-yl}-acetamide (**8**). Yield 67 %, mp 167-168ºC. ^{1}H NMR: δ 2.91 (s, 6H, 2*CH_3), 3.53 (dd, 1H, *J* = 19.2, 6.2 Hz, CH_2), 3.77 (s, 2H, CH_2), 4.01 (dd, 1H, *J* = 19.2, 11.3 Hz, CH_2), 5.56 (dd, 1H, *J* = 11.3, 6.2 Hz, CH), 6.76 (d, 2H, *J* = 8.5 Hz, arom.), 7.15-7.26 (m, 4H, arom.), 7.32 (t, 1H, *J* = 6.9 Hz, arom.), 7.39 (s, 1H, =CH), 7.49 (t, 2H, *J* = 7.9, arom.), 7.53 (m, 1H, arom.), 7.58-7.61 (m, 4H, arom.), 7.91 (dd, 2H, *J* = 9.0, 3.5 Hz, arom.), 10.84 (s, 1H, NH), 11.38 (s, 1H, NH). Anal.Calcd for $C_{35}H_{30}Cl_2N_6O_2S_2$: C, 59.91; H, 4.31; N, 11.98. Found: C, 59.98; H, 4.23; N, 11.85. ESI-MS m/z 700.7/702.7 $(M+H)^+$.

2-[2-(2,6-Dichlorophenylamino)-phenyl]-N-{5-[5-(4-methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydro-pyrazol-1-ylmethylene]-4-oxo-2-thioxothiazolidin-3-yl}-acetamide (**9**). Yield 69 %, mp 215-217ºC. ^{1}H NMR: δ 3.65 (s, 2H, CH_2), 3.76-3.79 (m, 4H, CH_2, OCH_3), 4.14 (dd, 1H, *J* = 18.7, 10.2 Hz, CH_2), 5.70 (dd, 1H, *J* = 10.2, 5.6 Hz, CH), 6.27-6.30 (m, 2H, arom.), 6.87 (d, 2H, *J* = 6.8 Hz, arom.), 6.99-7.06 (m, 4H, arom.), 7.15-7.19 (m, 2H, arom.), 7.31-7.39 (m, 3H, arom.), 7.50-7.52 (m, 3H, arom.), 7.94-7.99 (m, 2H, arom.), 8.11 (s, 1H, =CH), 10.62 (s, 1H, NH), 11.37 (s, 1H, NH). Anal.Calcd for $C_{38}H_{29}Cl_2N_5O_3S_2$: C, 61.79; H, 3.96; N, 9.48. Found: C, 61.72; H, 3.89; N, 9.55. ESI-MS m/z 737.7/739.7 $(M+H)^+$.

N-{5-[5-(4-Chlorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-4-oxo-2-thioxothiazolidin-3-yl}-2-[2-(2,6-dichlorophenylamino)-phenyl]-acetamide (**10**).

Yield 70 %, mp 179-180ºC. ^{1}H NMR: δ 3.41 (dd, 1H, *J* = 18.4, 6.4 Hz, CH_2), 3.69 (s, 2H, CH_2), 4.03 (dd, 1H, *J* = 18.4, 11.4 Hz, CH_2), 5.55 (dd, 1H, *J* = 11.4, 6.4 Hz, CH), 7.04-7.08 (m, 4H, arom.), 7.10 (s, 1H, arom.), 7.15 (d, 2H, *J* = 8.1 Hz, arom.), 7.16-7.18 (m, 2H, arom.), 7.22 (d, 2H, *J* = 8.1 Hz, arom.), 7.21 (d, 2H, *J* = 7.9 Hz, arom.), 7.26-7.34 (m, 5H, arom.), 8.03 (s, 1H, =CH), 10.50 (s, 1H, NH), 11.44 (s, 1H, NH). Anal.Calcd for $C_{37}H_{26}Cl_3N_5O_2S_2$: C, 59.80; H, 3.53; N, 9.42. Found: C, 59.92; H, 3.61; N, 9.32. ESI-MS m/z 742.1/744.1 $(M+H)^+$.

2-[2-(2,6-Dichlorophenylamino)-phenyl]-N-{5-[5-(4-fluorophenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-4-oxo-2-thioxothiazolidin-3-yl}-acetamide (**11**). Yield 64 %, mp 171-172ºC. ^{1}H NMR: δ 3.44 (dd, 1H, *J* = 18.8, 6.5 Hz, CH_2), 3.77 (s, 2H, CH_2), 4.01 (dd, 1H, *J* = 18.8, 11.4 Hz, CH_2), 5.65 (dd, 1H, *J* = 11.4, 6.5 Hz, CH), 7.04-7.09 (m, 5H, arom.), 7.15 (dd, 2H, *J* = 8.1, 4.0 Hz, arom.), 7.16-7.18 (m, 3H, arom.), 7.22 (d, 2H, *J* = 8.1 Hz, arom.), 7.25 (d, 2H, *J* = 7.9 Hz, arom.), 7.26-7.34 (m, 4H, arom.), 8.09 (s, 1H, =CH), 10.57 (s, 1H, NH), 11.33 (s, 1H, NH). Anal.Calcd for $C_{37}H_{26}Cl_2FN_5O_2S_2$: C, 61.16; H, 3.61; N, 9.64. Found: C, 61.22; H, 3.69; N, 9.53. ESI-MS m/z 725.6/727.6 $(M+H)^+$.

Pharmacology

Antitrypanosomal activity assay. Bloodstream forms of *Trypanosoma brucei brucei (Tbb)* strain 90-13 were cultured in HMI9 medium supplemented with 10 % FCS at 37°C under an atmosphere of 5 % CO_2 [22]. In all experiments, log-phase parasite cultures were harvested by centrifugation at 3,000 x *g* and immediately used. Drug assays were based on the conversion of a redox-sensitive dye (resazurin) to a fluorescent product by viable cells as previously described [23]. Drug stock solutions were prepared in pure DMSO. *Trypanosoma 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 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.

In vitro 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 [24-26]. 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*)] x 100 for concentrations for which *Ti* ³ *Tz*,

[(*Ti* – *Tz) / Tz*] x 100 for concentrations for which *Ti* < *Tz*.

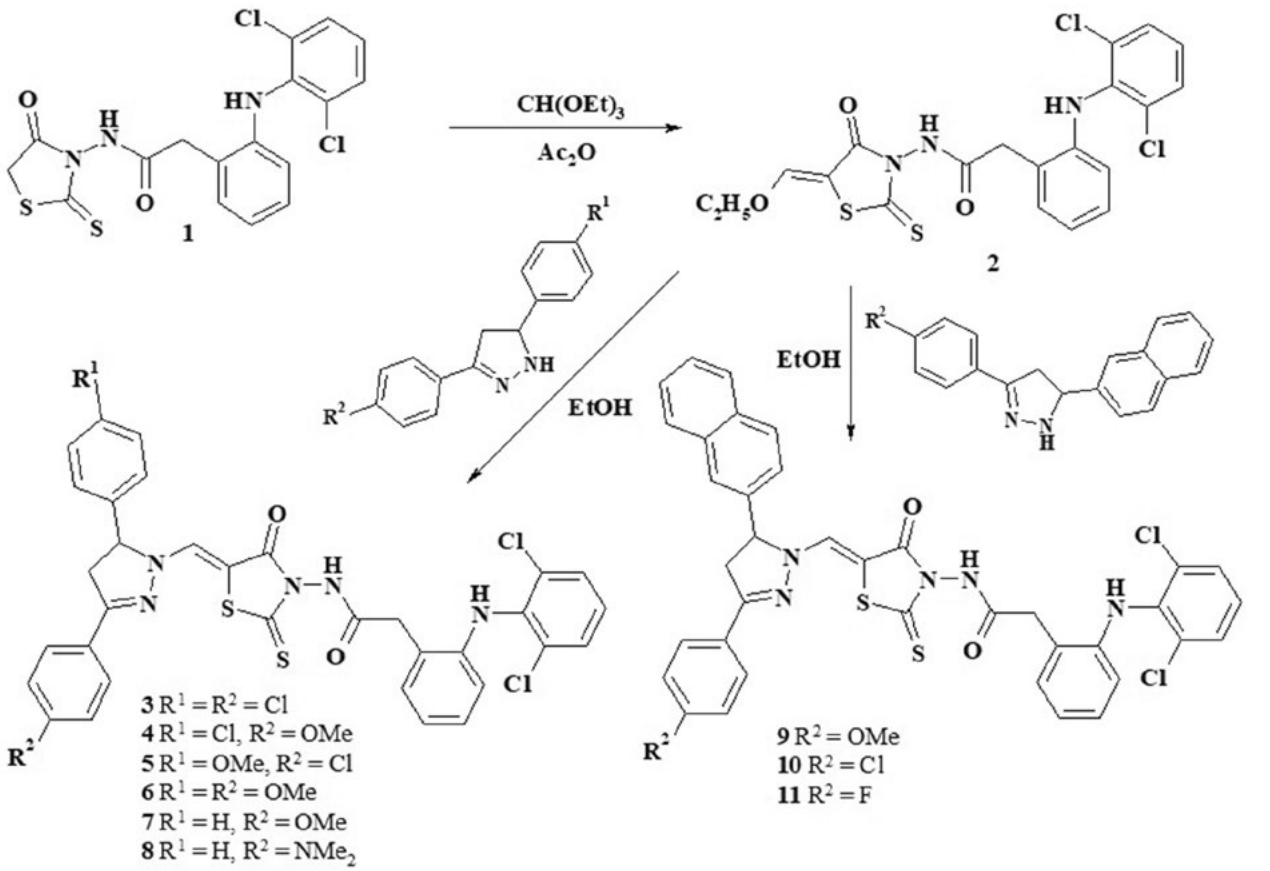
Dose response parameters (GI_{50}, TGI) were calculated for each compound. Growth inhibition of 50 % (GI_{50}) was calculated from [(*Ti* – *Tz*)/(*C* – *Tz*)] x 100 = 50, which is the drug concentration resulting in a 50 % lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells. The drug concentration resulting in total growth inhibition (TGI) was calculated as *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 ≤100 μM were declared to be active.

Results and Discussion

Chemistry

Target compounds were synthesized using synthetic protocols including stage of 2-[2-(2,6-dichlorophenylamino)-phenyl]-*N*-(4-oxo-2-thioxothiazolidin-3-yl)-acetamide **1** formation based on the reaction between thiocarbonyl-bis-thioglycolic acid and 2-[2-(2,6-dichloroanilino)phenyl]acetohydrazide as described previously [27]. Further condensation of **1** with triethyl orthoformate yielded 2-[2-(2,6-dichlorophenylamino)-phenyl]-*N*-(5-ethoxymethylene-4-oxo-2-thioxothiazolidin-3-yl)-acetamide **2**. 5-Ethoxy-4-thiazolidinone **2** was converted into appropriate 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones **3-11** *via* aminolysis reaction with different 3,5-diaryl-4,5-dihydro-1*H*-pyrazoles in ethanol medium.

The structures of the synthesized compounds were confirmed by elemental analysis and spectroscopic data (^{1}H NMR and LCMS). In ^{1}H NMR spectra the characteristic secondary amine proton of diclofenac fragment appears as a singlet at δ ~10.50–11.35 ppm and methylene protons assigned as a singlet at δ ~3.65–3.85 ppm, respectively. The pyrazoline fragment of **3-11** shows characteristic patterns of an AMX system for CH_2−CH protons. The chemical shifts of the protons H_A, H_M, and H_X have been assigned as a doublet of doublets at δ ≈ 3.39−3.79, δ ≈ 3.97−4.14, and δ ≈ 5.50−5.74 with corresponding coupling constants of J_{AM} = 18.0−19.2, J_{AX} = 10.2−11.4, and J_{MX} = 5.6−6.8 Hz, respectively. The CH protons of the methylylidene group (= CH) of synthesized compounds appear as a singlet at δ 7.39–8.11 ppm.



Scheme 1

In vitro evaluation of the anticancer activity

The synthesized 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones **7** and **9** were 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 were performed according to the US NCI protocol, which was described elsewhere (see e.g. http://dtp.nci.nih.gov) [23-26]. 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 2-[2-(2,6-dichlorophenylamino)-phenyl]-*N*-{5-[5-(4-methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-4-oxo-2-thioxothiazolidin-3-yl}-acetamide **9** was found to be effective with the average cell growth indices (GP_{mean}) of 22.40 %. Thus, compound **9** demonstrated

Table 1. **Anticancer screening data in concentration 10^{-5} M.**

Comp. NSC	Mean growth, %	Range of growth, %	Most sensitive cell line growth, % (*cancer line*/type)
7 789612	99.30	79.35 to 121.08	79.35 (UO-31/renal cancer)
9 808312	22.40	-49.77 to 69.97	6.24 (CCRF-CEM/ leukemia) -27.37 (HL-60(TB)/ leukemia) 10.63 (K-562/leukemia) -10.09 (RPMI-8226/leukemia) 9.42 (NCI-H460/NSCLC) -15.38 (NCI-H522/ NSCLC) -16.88 (SF-539/CNS Cancer) -15.72 (SNB-75/CNS Cancer) -25.06 (M14/Melanoma) -49.77 (MDA-MB-435 /Melanoma) 2.15 (OVCAR-3 /Ovarian Cancer) 9.39 (NCI/ADR-RES/Ovarian Cancer) -0.47 (A498 /Renal Cancer) -8.96 (RXF 393 /Renal Cancer) 7.11 (MDA-MB-468/Breast Cancer)

high cytotoxic effect on leukemia HL-60(TB) and RPMI-8226 (GP = – 27.37 and – 10.09 %, respectively), non-small cell lung cancer NCI-H522 (GP = – 15.38 %), CNS cancer SF-539 and SNB-75 (GP = – 16.88 and – 15.72 %, respectively), melanoma M14 and MDA-MB-435 (GP = – 25.06 and – 49.77 %, respectively) cell lines.

Finally, compound **9** possessed considerable activity against all 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) [23-26]. 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. Three antitumor activity dose-response parameters were calculated 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. 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. Compound **9** showed a broad spectrum of growth inhibition activity against tested human tumor cells with average GI_{50} and TGI values 0.71/1.09 and 82.95/28.46μM, respectively (Table 2). The selectivity index (SI) obtained by dividing the full panel MG-MID (μM) of the compound **9** by their individual subpanel MG-MID of cell line (μM) was considered as a measure of compound's selectivity. The compound **9** in the present study was found to be nonselective at

both the GI_{50} and TGI levels (selectivity indexes 0.12-5.07/0.10-5.19 and 1.21-148.12/0.29-53.69, respectively) (Table 2). However, the mentioned derivative demonstrated a certain selectivity profile toward some individual cell lines at TGI level. Thus, selectivity indices were 28.80-148.12/6.68-53.69 for RPMI-8226 (leukemia), SF-539 and SNB-75 (CNS cancer), MDA-MB-435 (melanoma), OVCAR-3 (ovarian cancer), A498, RXF 393 and UO-31 (renal cancer).

Antitrypanosomal activity

The antitrypanosomal activity of the novel 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones **3, 4, 6, 7, 9-11** was studied in *in vitro* assay towards *Trypanosome brucei brucei* (*Tbb*). The IC_{50} values were calculated based on at least three independent experiments. In general, the synthesized compounds displayed slight inhibition on growth of the tested parasites (Table 3). Hovewer, among the tested derivatives the most active compounds were found to be **3** and **6** with IC_{50} values of 15.0 and 17.2 μM, respectively. The SAR study revealed that the level of antitrypanosomal activity of 5-(3,5-diaryl-4,5-dihydropyrazol-1-ylmethylene)-2-thioxothiazolidin-4-ones depends on substituent at N3 of thiazolidinone core. Noteworthy,

Table 2. **Influence of compound 9 on the growth of individual tumor cell lines in two independent assays.**

Disease	Cell line	GI_{50}, μM	SI (GI_{50})	TGI, μM	SI (TGI)	LC_{50}, μM	SI (LC_{50})
Leukemia	CCRF-CEM	0.14/0.39	5.07/2.79	>100.0/>100.0	-/-	>100.0/>100.0	-/-
	HL-60(TB)	0.32/0.50	2.21/2.18	1.23/6.77	67.43/4.20	>100.0/>100.0	-/-
	K-562	0.35/0.45	2.02/2.42	>100.0/>100.0	-/-	>100.0/>100.0	-/-
	MOLT-4	0.37/0.59	0.91/1.84	>100.0/>100.0	-/-	>100.0/>100.0	-/-
	RPMI-8226	0.28/0.33	2.53/3.30	0.74/4.26	112.09/6.68	>100.0/>100.0	-/-
	SR	0.19/0.30	3.73/3.63	-/>100.0	-/-	>100.0/>100.0	-/-
	MG_MID	**0.27/0.42**	**2.62/2.59**	**50.32/68.50**	**1.64/0.41**	**>100/>100**	**-/-**
NSC lung cancer	A549/ATCC	0.42/0.60	1.69/1.81	>100.0/22.2	-/1.28	>100.0/>100.0	-/-
	EKVX	2.28/0.92	0.31/1.18	>100.0/37.8	-/0.75	>100.0/>100.0	-/-
	HOP-62	0.86/1.07	0.82/1.01	>100.0/24.9	-/1.14	>100.0/>100.0	-/-
	HOP-92	0.74/2.15	0.95/0.50	>100.0/28.5	-/0.99	>100.0/>100.0	-/-
	NCI-H266	2.30/0.56	0.30/1.94	>100.0/10.4	-/2.73	>100.0/58.1	-/1.27
	NCI-H23	0.76/0.82	0.93/1.32	>100.0/21.9	-/1.29	>100.0/88.1	-/0.84
	NCI-H322M	0.44/0.74	1.61/1.47	>100.0/65.6	-/0.43	>100.0/>100.0	-/-
	NCI-H460	0.38/0.39	1.86/2.79	>100.0/11.5	-/2.47	>100.0/45.1	-/1.64
	NCI-H522	0.26/0.37	2.73/2.94	-/4.76	-/5.97	>100.0/>100.0	-/-
	MG_MID	**0.93/0.84**	**0.76/1.29**	**>100/25.28**	**-/1.12**	**>100.87.92**	**-/0.84**
Colon cancer	COLO 205	1.89/-	0.37/-	>100.0/-	-/-	>100.0/-	-/-
	HCC-2998	1.24/1.55	0.57/0.70	>100.0/11.1	-/2.56	>100.0/75.9	-/0.97
	HCT-116	0.44/0.51	1.61/2.13	>100.0/15.7	-/1.81	>100.0/53.8	-/1.37
	HCT-15	0.44/0.49	1.61/2.22	>100.0/42.4	-/0.67	>100.0/>100.0	-/-
	HT29	2.29/2.31	0.31/0.47	>100.0/6.91	-/4.11	>100.0/26.6	-/2.79
	KM12	0.38/0.35	1.86/3.11	>100.0/11.2	-/2.54	>100.0/40.2	-/1.84
	SW-620	0.41/0.41	1.73/2.65	>100.0/27.7	-/1.02	>100.0/>100.0	-/-
	MG_MID	**1.01/0.93**	**0.70/1.17**	**>100/19.16**	**-/1.48**	**>100/66.08**	**-/1.12**

Continued Table 2

CNS cancer	SF-268	0.98/0.66	0.72/1.65	>100.0/23.8	-/1.19	>100.0/90.5	-/0.82
	SF-295	1.17/0.45	0.61/2.42	>100.0/10.7	-/2.65	>100.0/39.5	-/1.87
	SF-539	0.25/0.31	2.84/3.51	0.75/1.67	110.6/17.04	>100.0/20.6	-/3.60
	SNB-19	0.51/0.79	1.39/1.37	>100.0/22.0	-/1.29	>100.0/>100.0	-/-
	SNB-75	0.20/0.32	3.55/3.40	0.86/2.26	96.45/12.59	>100.0/24.5	-/3.02
	U251	0.39/0.40	1.82/2.72	>100.0/13.5	-/2.10	>100.0/37.3	-/1.99
	MG_MID	**0.58/0.48**	**1.22/2.27**	**66.93/12.32**	**1.23/2.31**	**>100/52.06**	**-/1.42**
Melanoma	LOX IMVI	0.55/0.55	1.29/1.98	>100.0/29.1	-/0.97	>100.0/>100.0	-/-
	MALME-3M	0.62/2.88	1.14/0.37	>100.0/24.0	-/1.18	>100.0/70.6	-/1.05
	M14	0.34/0.47	2.08/2.31	2.88/10.1	28.80/2.81	>100.0/92.0	-/0.80
	MDA-MB-435	0.22/0.21	3.22/5.19	0.56/0.53	148.12/53.69	7.21/2.53	13.65/29.33
	SK-MEL-2	-/2.95	-/0.36	>100.0/18.8	-/1.51	>100.0/>100.0	-/-
	SK-MEL-28	5.86/3.37	0.12/0.32	>100.0/25.0	-/1.13	>100.0/82.5	-/0.89
	SK-MEL-5	0.46/0.50	1.54/2.18	>100.0/13.6	-/2.09	>100.0/41.3	-/1.79
	UACC-257	1.37/10.9	0.51/0.1	>100.0/>100.0	-/-	>100.0/>100.0	-/-
	UACC-62	0.49/0.74	1.44/1.47	>100.0/19.2	-/1.48	>100.0/59.4	-/1.24
	MG_MID	**1.10/2.50**	**0.64/0.43**	**78.16/26.70**	**1.06/1.06**	**89.69/72.03**	**1.09/1.03**
Ovarian cancer	IGROV1	0.48/0.71	1.47/1.53	>100.0/17.7	-/1.60	>100.0/59.1	-/1.25
	OVCAR-3	0.34/0.33	2.08/3.30	>100.0/0.95	-/29.9	>100.0/20.9	-/3.55
	OVCAR-4	0.82/1.74	0.86/0.62	>100.0/23.0	-/1.23	>100.0/65.1	-/1.14
	OVCAR-5	0.83/3.63	0.85/0.30	>100.0/20.8	-/1.36	>100.0/66.0	-/1.12
	OVCAR-8	0.50/0.94	1.42/1.15	>100.0/>100.0	-/-	>100.0/>100.0	-/-
	NCI/ADR-RES	0.40/0.43	1.77/2.53	>100.0/11.9	-/2.39	>100.0/>100.0	-/-
	SK-OV-3	0.84/-	0.84/-	>100.0/-	-/-	>100.0/-	-/-
	MG_MID	**0.60/1.29**	**1.18/0.84**	**>100/29.05**	**-/0.97**	**>100/68.51**	**-/1.08**
Renal cancer	786-0	0.49/0.51	1.44/2.13	>100.0/14.5	-/1.96	>100.0/57.8	-/1.28
	A498	0.30/-	2.36/-	0.99/-	83.78/-	>100.0/-	-/-
	ACHN	0.72/0.64	0.98/1.70	>100.0/51.1	-/0.55	>100.0/>100.0	-/-
	CAKI-1	0.33/0.45	2.15/2.42	>100.0/16.0	-/1.77	>100.0/50.8	-/1.46
	RXF 393	0.22/0.35	3.22/3.11	0.83/1.61	99.93/17.67	>100.0/22.3	-/3.32
	SN12C	0.49/0.85	1.44/1.28	>100.0/24.0	-/1.18	>100.0/>100.0	-/-
	TK-10	-/3.57	-/0.30	>100.0/43.2	-/0.65	>100.0/>100.0	-/-
	UO-31	0.29/0.42	2.44/2.59	>100.0/3.02	-/9.42	>100.0/23.4	-/3.17
	MG_MID	**0.35/1.13**	**2.02/0.96**	**75.21/21.91**	**1.10/1.29**	**>100/64.9**	**-/1.14**
Prostate cancer	PC-3	0.66/0.76	1.07/1.43	>100.0/29.1	-/0.97	>100.0/>100.0	-/-
	DU-145	0.38/0.42	1.86/2.59	68.4/10.8	1.21/2.63	>100.0/32.8	-/2.26
	MG_MID	**0.52/0.59**	**1.36/1.84**	**84.2/19.95**	**0.98/1.42**	**>100/66.4**	**-/1.11**
Breast cancer	MCF7	0.36/0.36	1.97/3.02	>100.0/25.5	-/1.11	>100.0/>100.0	-/-
	MDA-MB-231/ ATCC	1.61/1.60	0.44/0.68	>100.0/19.7	-/1.44	>100.0/70.3	-/1.05
	HS 578T	0.35/0.48	2.02/2.27	>100.0/14.2	-/2.00	>100.0/>100.0	-/-
	BT-549	0.57/0.80	1.24/1.36	>100.0/18.5	-/1.53	>100.0/52.1	-/1.42
	T-47D	0.50/1.23	1.42/0.88	>100.0/97.6	-/0.29	>100.0/>100.0	-/-
	MDA-MB-468	0.56/0.52	1.26/2.09	>100.0/11.7	-/2.43	>100.0/62.4	-/1.18
	MG_MID	**0.65/0.83**	**1.09/1.31**	**>100/31.20**	**-/0.91**	**>100/80.8**	**-/0.91**
MG_MID		**0.71/1.09**		**82.95/28.46**		**98.45/74.23**	

the diclofenac moiety in the N3 position of the basic heterocycle was adverse on activity against *Trypanosoma* species compared to previously described substituted pyrazoline-thiazolidinone hybrid molecules [19, 28].

Table 3. **Antitrypanosomal activity of the tested compounds**

Compound	*Tbb* IC_{50}, μM
3	15.0 ± 0.12
4	29.0 ± 0.45
6	17.2 ± 0.15
7	72.6± 0.43
9	49.3 ± 0.35
10	67.3 ± 0.47
11	68.8 ± 0.41
Pentamidine, nM	2.40 ± 0.06

Conclusion

We herein report the synthesis, LCMS and ^{1}H NMR spectroscopic data and pharmacological evaluation of a novel series of rhodanine-pyrazoline hybrid molecules with a diclofenac moiety (**3**-**11**). Anticancer activity study of the synthesized compounds allowed identification of the most active derivative 2-[2-(2,6-dichlorophenylamino)-phenyl]-*N*-{5-[5-(4-methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-ylmethylene]-4-oxo-2-thioxo-thiazolidin-3-yl}-acetamide **9** with the mean GI_{50} = 0.71/1.09 μM and TGI = 82.95/28.46 μM. The tested compounds display slight antitrypanosomal activity against *Trypanosome brucei brucei* (*Tbb*). The biological tests of such thiazolidine-pyrazoline hybrids with diclofenac moiety in the molecules revealed the necessity for further evaluation of anticancer, antitrypanosomal activities for the design of novel drug-like molecules with better pharmacological profiles.

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Синтез та оцінка біологічної активності роданін-піразолінових гібридних молекул з 2-(2,6-дихлорофеніламіно)фенілацетамідним фрагментом

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Мета. На основі реакцій гетероциклізації та амінолізу здійснити синтез нових роданін-піразолінових гібридних молекул з фрагментом диклофенаку в положенні 3 для скринінгу їх протипухлинної та антитрипаносомної активності. **Методи.** Органічний синтез, спектроскопія ЯМР, фармакологічний скринінг. **Результати.** Взаємодією гідразиду 2-(2,6-дихлорофеніламіно)-фенілацетатної кислоти з тіокарбоніл-бістіогліколевою кислотою в середовищі етанолу синтезовано похідне роданіну з фрагментом протизапального засобу диклофенаку в положенні 3. Враховуючи наявність активної метиленової групи в положенні 5 проведено подальшу модифікацію з утворенням 5-етоксиметиленроданіну, який в умовах реакції амінолізу з різноманітними 3,5-діарил-4,5-дигідро-1*H*-піразолами трансформований у серію відповідних 5-(3,5-диарил-4,5-дигідропіразол-1-ілметилен)-2-тіоксотіазолідин-4-онів. Скринінг протипухлинної активності дозволив ідентифікувати високоактивну сполуку **9** з середніми значеннями GI_{50} = 0.71/1.09 μM та TGI = 82.95/28.46 μM на 60 лініях ракових клітин (програма DTP NCI). Синтезовані піразолін-тіазолідинові гібридні молекули з фрагментом диклофенаку у структурі не проявили помітної антитрипаносомної активності відносно збудників *Trypanosoma brucei brucei (Tbb)*. **Висновки.** Синтезовані 5-(3,5-диарил-4,5-дигідропіразол-1-ілметилен)-2-тіоксотіазолідин-4-они з фрагментом диклофенаку у структурі є перспективною молекулярною платформою для розробки нових високоактивних та малотоксичних сполук як потенційних лікарських засобів.

Ключові слова: синтез, 2-тіоксо-4-тіазолідинон, диклофенак, спектральні характеристики, протипухлинна активність, антитрипаносомна активність

Синтез и оценка биологической активности роданин-пиразолиновых гибридных молекул с 2-(2,6-дихлорофениламино) фенилацетамидным фрагментом

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Цель. Основываясь на реакциях гетероциклизации и аминолиза осуществить синтез новых роданин-пиразолиновых гибридных молекул с фрагментом диклофенака в положении 3 для скрининга противоопухолевой и антитрипаносомной активности. **Методы.** Органический синтез, спектроскопия ЯМР, фармакологический скрининг. **Результаты.** Взаимодействием гидразида 2-(2,6-дихлорофениламино)-фенилацетатной кислоты с тиокарбонил-бис-тиогликолевой кислотой в среде этанола синтезировано производное роданина с фрагментом противовоспалительного средства диклофенака в положении 3. Учитывая наличие активной метиленовой группы в положении 5, проведено дальнейшую модификацию с образованием 5-етоксиметиленроданина, который в условиях реакции аминолиза с различными 3,5-диарил-4,5-дигидро-1*H*-пиразолами трансформирован в серию соответствующих 5-(3,5-диарил-4,5 дигидропиразол-1-илметилен)-2-тиоксотиазолидин-4-онов. Скрининг противоопухолевой активности позволил идентифицировать высокоактивное соединение **9** из средними значениями GI_{50} = 0.71/1.09 μM и TGI = 82.95/28.46 μM на 60 линиях раковых клеток (программа DTP NCI). Синтезированные пиразолин-тиазолидиновые гибридные молекулы с фрагментом диклофенака в структуре не проявили заметной антитрипаносомной активности в отношении возбудителей *Trypanosoma brucei brucei (Tbb)*. **Выводы.** Синтезированные 5-(3,5-диарил-4,5 дигидропиразол-1-илметилен)-2-тиоксотиазолидин-4-оны с фрагментом диклофенака в структуре являются перспективной молекулярной платформой для разработки новых высокоактивных и малотоксичных соединений как потенциальных лекарственных средств.

Ключевые слова: синтез, 2-тиоксо-4-тиазолидинон, диклофенак, спектральные характеристики, противоопухолевая активность, антитрипаносомная активность.

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