SUBSTITUTED 1,3-PHENYL(PYRIDYL) PROPENONES AND DERIVATIVES WITH THIOSEMICARBAZIDIC GROUPS. STRUCTRURE – (HL-60) ANTILEUKEMIA ACTIVITY RELATIONSHIP

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Abstract. 3-(4-(Dimethylamino)phenyl-1-(4-isothiocyanatophenyl)prop-2-en-1-one was obtained from the corresponding N,N-dimethylthyoureas by elimination of dimethylamine at heating with gaseous hydrogen chloride in chloroform and 1-(4-isothiocyanatophenyl)-3-(pyridin-2-il)prop-2-en-1-one by treating 1,1-dimethyl-3-(4-(3-(pyridin-2-il)-acryloyl)-phenyl)thyourea with acetic anhydride. The difference in the reactivity of the groups >C=O and NCS in the synthesis with hydrazine hydrate and its derivatives allows the synthesis of some 1,3-disubstituted propenones with thiosemicarbazide groups (4- and 1,4-disubstituted) in good yields. From 4-substituted thiosemicarbazides and 2-formilpyridine thiosemicarbazones were obtained. In the case of some derivatives, the propenone group in the reaction with hydrazine hydrate allows the formation of pyrazole derivatives. All obtained compounds were investigated for antileukemia activity. It was found that this activity is more pronounced for thiosemicarbazide derivatives with two pyridine nuclei at concentrations 10^{-5}-10^{-7} mol/L.

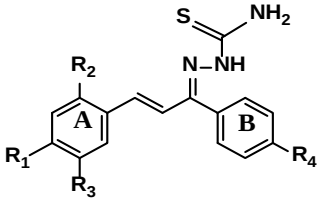
Keywords: chalcones, isothiocyanatopropenones, thioureas, antileukemia activity.

Introduction

3-(4-(Dimethylamino)phenyl-1-(4-isothiocyanatophenyl)prop-2-en-1-one was obtained from the corresponding amine treated with triphosgene [1] in 80% yield. The authors [2] obtained this chalcone by the elimination of dimethylamine from 3-(4-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl)-1,1-dimethylthiourea at heating with acetic anhydride, with an yield of 92%. This compound shows high fluorescence and can be used as marker for proteins [3] and for the synthesis of luminescence polymer nanocomposites [4].

Thiosemicarbazides 4-and 1,4-disubstituited can be obtained at the addition of hydrazine and its derivatives to isothiocyanates [5,6], or directly from N-aril-N,N-dimethylthiourea [7]. The purpose of this study was to obtain biologically active compounds.

In the paper [8], the anticancer activity (for 5 types of cancer) was investigated for a class of chalcones with different substituents (H, CH_3, OH, OCH_3, $N(CH_3)_2$, Cl) for both aromatic nuclei (A and B). In some cases the chalcones with OCH_3 and $N(CH_3)_2$ groups show higher anticancer activity. The thiosemicarbazones of some chalcones show antitumor activity for HepG2 cell line [9]. The authors [10] investigated the anticancer activity for some thiosemicarbazones with the structures depicted in Figure 1.



a) R_1=F, R_2=H, R_3=H, R_4=H
b) R_1=Cl, R_2=H, R_3=H, R_4=H
c) R_1=Br, R_2=H, R_3=H, R_4=H
d) R_1=OMe, R_2=H, R_3=H, R_4=H
e) R_1=H, R_2=H, R_3=F, R_4=H
f) R_1=H, R_2=H, R_3=OMe, R_4=H
g) R_1=H, R_2=H, R_3=NO_2, R_4=H
h) R_1=H, R_2=F, R_3=H, R_4=H
i) R_1=H, R_2=Cl, R_3=H, R_4=H
j) R_1=H, R_2=Br, R_3=H, R_4=H
k) R_1=H, R_2=Me, R_3=H, R_4=H
l) R_1=H, R_2=OMe, R_3=H, R_4=H
m) R_1=H, R_2=NO_2 R_3=H, R_4=H
n) R_1=H, R_2=Ph, R_3=H, R_4=H
o) R_1=H, R_2=OCH_2Ph, R_3=H, R_4=H
p) R_1=H, R_2=H, R_3=H, R_4=H
q) R_1=H, R_2=H, R_3=H, R_4=Br
r) R_1=H, R_2=H, R_3=H, R_4=Me
s) R_1=H, R_2=H, R_3=H, R_4=OMe
t) R_1=H, R_2=H, R_3=H, R_4=Cl
u) R_1=H, R_2=H, R_3=Cl, R_4=Cl
v) R_1=H, R_2=F, R_3=H, R_4=Br
w) R_1=Cl, R_2=H, R_3=H, R_4=oMe

Figure 1. The structure of chalcone thiosemicarbazide derivatives (a-w).

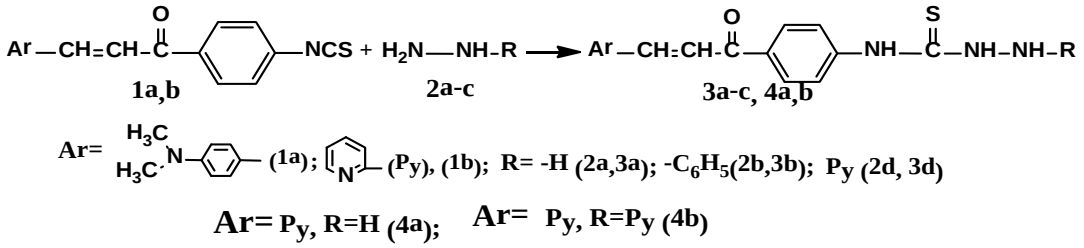
It was demonstrated that the anticancer activity depends on the nature and the position of the substituents in the structure. The 1,3-aril(heteryl-2-prop-1-ones chalcones at heating in a basic medium allow the formation of 1-thiocarbamoil-3-phenyl-5-heteroaril-2-pyrazoline with anticonvulsant and antidepressant properties [11].

The (1,3-aril(heteryl)propen-2-one) chalcones with thiosemicarbazidic groups (4-and 1,4-disubstituted) and respectively thiosemicarbazones are lacking in the literature and they became our object of study.

Results and discussion

Chemistry

Introduction of groups 4- and 1,4-thiosemicarbazide in chalcones structure was performed by treating isothiocianato-1,3-prop-2-one **1a, b** with hydrazine or with their derivatives **2a-d** following the Scheme 1.

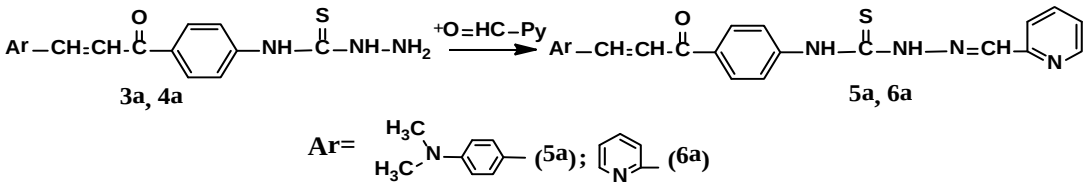


Scheme 1. General synthesis of chalcone thiosemicarbazide derivatives (3a-c, 4a,b).

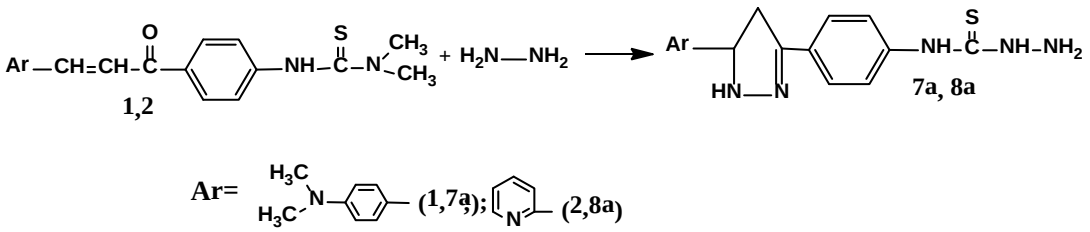
Taking into account the high activity of NCS group in the reaction with nucleophile agents, the synthesis was realized at room temperature with a molar ratio of reagents of 1:1, to exclude the participation of the carbonyl group at condensation with hydrazine hydrate and its derivatives. Benzene was used as solvent, from which the hydrazinecarbothioamides **3a-c** and **4a,b** crystallized during the synthesis. Hydrazinecarbothioamides **3a** and **4a** which condense at heating were filtrated and washed first with benzene and after with water. They were used without further recrystallization. The other compounds can be recrystallized from corresponding solvents in a yield of 60-92%.

The synthesis of N-(4-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl)-2-(pyridin-2-ylmethylene)-hydrazine-carbothioamide **5a** and N-(4-(3-(pyridin-2-yl)acryloyl)phenyl)-2-(pyridin-2-ylmethylene)- hydrazinecarbothioamide **6a** (Scheme 2) is more efficient in the presence of acetic acid, when the solution of 2-formylpyridine is in excess. The solution of thiosemicarbazides **3a** or **4a** was added droplet by droplet. Thus, the interaction of chalconic >C=O group with thiosemicarbazidic group was excluded. The yields of thiosemicarbazones **5a** and **6a** reached a value of 83% and 77% (Scheme 2).

4,5-Dihydro-1H-pyrazol-3-yl)phenyl)hydrazinecarbothioamides **7a** and **8a** were obtained through the sequence of reactions that is indicated in Scheme 3.



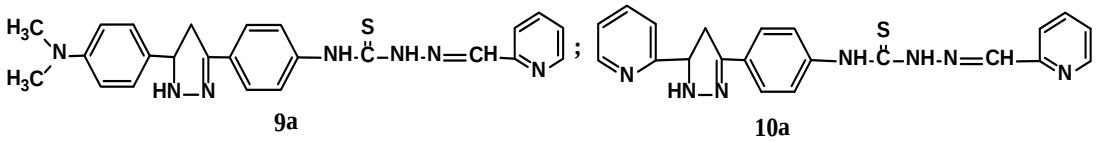
Scheme 2. Synthesis of hydrazinecarbothioamide (5a, 6a).



Scheme 3. Synthesis of chalcone derivatives (1, 7a, 2, 8a).

First the N,N-dimethylthioureas 1,2 with hydrazine at room temperature transforms in hydrazones in pyridine, which without being isolated at heating it will cyclise in pyrazole derivatives [7]. In parallel, dimethylamine is substituted by hydrazine [11] with the formation of compounds **7a** and **8a** in good yields. Compound **7a** was also obtained by an alternative method, from isothiocyanatophenylprop-2-en-1-one **1a**, pyridine and hydrazine at room temperature, after that the mixture was heated. It could be possible that the same intermediate is formed, which is transformed into the final product **7a**.

Compounds **9a** and **10a** (Figure 2) were obtained from **7a** and **8a** at heating in ethanol with 2-formylpyridine.



9a **10a**

Figure 2. Structure of chalcone derivatives (9a, 10a).

Biological Activity

Antiproliferative Activity of Human Leukemia HL-60 Cells

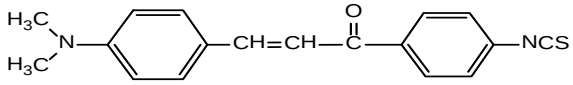
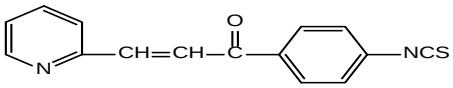
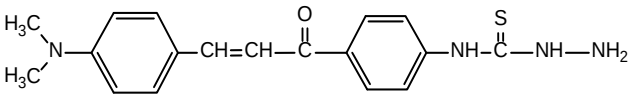
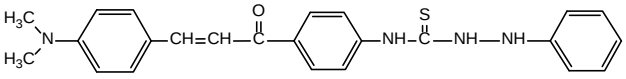
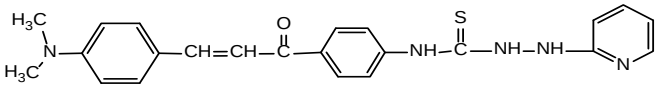
For all obtained compounds the antileukemia activity was investigated. It is more pronounced for thiosemicarbazide derivatives with two pyridine nuclei at concentrations 10^{-5}-10^{-7} mol/L (see Table 1).

The aryl isothiocyanates with different substituents in their structure are inactive against leukemia [12]. Isothiocyanatochalcone **1a** and 1-(4-isothiocyanatophenyl)-3-(pyridine-2-yl)prop-2-en-1-one **1b** are also inactive. Similarly, the N-phenylhydrazinecarbothioamide **11a** is inactive. It was found that the modification of the –NCS fragment in compounds **1a** and **1b** by the addition of the hydrazine and its derivatives, leads to anticancer activity for all the chalcones **3a-d**. Chalcone **3c**, which contain residues of pyridine in the first position of the tiosemicarbazidic fragment, shows an inhibition of the antileukemia of 92% at concentration of 10^{-5} mol/L. It can be concluded that the introduction of the fragment $(CH_3)_2N$-C_6H_4-CH=CH-CO- or Py-CH=CH-CO- in the molecule of N-phenylhydrazinecarbothioamide **11a** plays an important role to increase the anticancer activity. This activity increases when the fragment $(CH_3)_2N$-C_6H_4- of the chalcone **3c** is replaced by rest of pyridine, propenone **4b** (100%, C =10^{-5} mol/L).

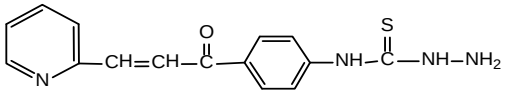
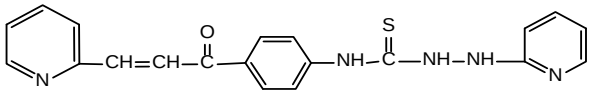
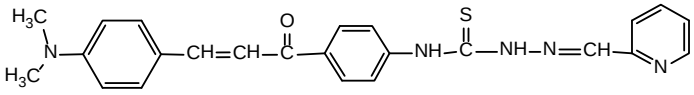
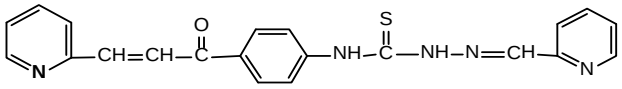
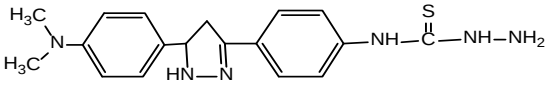
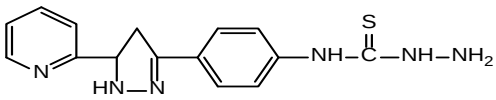
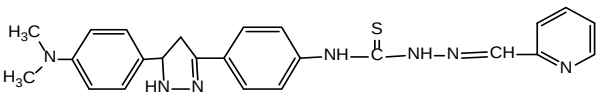
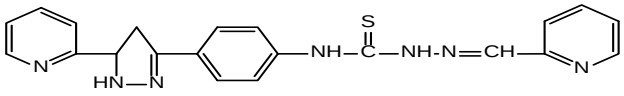
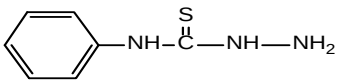
To highlight the role of the propenonic group on the anticancer activity on the propenones **3a**, **4a**, **5a** and **6a** the fragment -CH=CH-CO- was transformed in pyrazole heterocycle. For all obtained and studied samples **7a**, **8a**, **9a** and **10a** a sudden decrease of anticancer activity was observed (see Table 1). It was identified that for compound **10a** with two pyridine nuclei the inhibitor activity decrease slower when is diluted and achieves ~60% at concentration of 10^{-7} mol/L. The high anticancer activity for compound **10a** can be explained by the formation of the strong hydrogen bonds between the inhibitor and the nucleic acid of the cancer cells [13].

Table 1

Antiproliferative activity of compounds on human leukemia (HL-60) cells at three concentrations.

No	*Compound*	*Inhibition of cell proliferation, (%)*		
		Concentration, mol/L		
		10^{-5}	10^{-6}	10^{-7}
1a		0	0	0
1b		0	0	0
3a		34.2	26.7	19.2
3b		52.2	6.0	0
3c		92.4	34.2	22.0

Continuation of the Table 1

4a		48.9	21.6	14.3
4b		100.0	25.1	24.6
5a		55.9	26.0	11.8
6a		100	46.7	28.5
7a		20.8	11.5	6.0
8a		30.4	24.1	20.5
9a		46.8	20.5	9.4
10a		-	62.5	58.1
11a		0	0	0

Conclusions

Ten new propenones and thiosemicarbazidic groups have been synthesized and characterized. The IR, ^{1}H-NMR and ^{13}C-NMR data were successfully used to elucidate the formation of the structure of compounds. All obtained compounds were investigated for antileukemia activity. It was found that this activity is more pronounced for thiosemicarbazide derivatives with two pyridine nuclei at concentrations 10^{-5}-10^{-7} mol/L.

Experimental

The structure of compounds **1a**, **b**, **3a-c**, **4a**, **b**, **5a**, **6a**, **7a**, **8a, 9a** and **10a** was confirmed by elemental and spectral analysis (^{1}H and ^{13}C NMR). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III-400 spectrometer at room temperature. All chemical shifts (^{1}H, ^{13}C) are given in ppm versus $SiMe_4$ using DMSO-d_6 as solvent. Elemental analyses (C, H and N) were performed on an Elemental Analyzer Vario EL (III). Compounds **3a** and **4a** form insoluble in organic solvents polycondensed compounds at heating until the melting point. The melting points were determined with a Melting point meter A. KRUSS OPTRONIC Germany KSP-1N 90-26V/Al.

3-(4-(Dimethylamino)phenyl-1-(4-isothiocyanatophenyl)prop-2-en-1-one 1a. 0.7 g (0.002 mol) of 3-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)-1,1-dimethylthiourea **1** and 6 mL of chloroform were placed in one flask. After this, the solution was cooled and gaseous hydrogen chloride was passed through it. Once the mass of the mixture increase with 0.15 g, the ampoule was welded and heated at 70^{o}C for 3 hours. The resulting product was neutralized to pH=7, the organic solution was dried with Na_2SO_4 and a part of the solvent was distilled. The final product was purified by chromatography on Silicagel (eluent hexane/benzene, 1/5). It was obtained 0.54 g (88%) of propenone **1a** m.p. 136-138^{o}C which corresponds with the literature results [2].

Elemental analysis and NMR data: Calculated of $C_{18}H_{16}N_2OS$ (**1a**), %: C-70.26, H-5.28, N-9.26. Found, %: C-70.10, H-5.23, N-9.08. **^{1}H-NMR** (DMSO-d_6), ppm: 3.02 (s, 6H, $N(CH_3)_2$), 6.74-8.20 (m, 10H, =CH and C_6H_4). **^{13}C-NMR** (DMSO-d_6), ppm: 187.82 (C=O), 181.39 (C=S), 152.42 (C-N), 145.14 (-C_6H_4-**CH=**), 137.11 (-C_6H_4-**N=C=S**), 145.14, 134.39, 131.45, 131.15, 129.46, 126.67, 122.59, 122.01, 111.54, 40.65, 40.44.

N-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)hydrazinecarbothioamide 3a. The mixture formed by 0.61 g (0.002 mol) 3-(4-(Dimethylamino)phenyl-1-(4-isothiocyanatophenyl)prop-2-en-1-one **1a**, 0.13 g (0.0025 mol) hydrazine hydrate and 5 mL of ethanol was kept at room temperature for 30 min. After, it was heated at 40°C for 5 min. The CCM (silufol) show the total consumption of isothiocyanatochalcone **1a**. The reacting mixture was cooled and the resulting crystals were filtrated and washed with ethanol. Yield 0.63 g (92 %) of hydrazinecarbothioamide **3a**, m.p. >155°C (is polycondensatione).
Elemental analysis and NMR data: Calculated of $C_{18}H_{20}N_4OS$ (**3a**), %: C-63.50, H-5.92, N-16.47. Found, %: C-63.53, H-6.01, N-16.84. **^{1}H-NMR** (DMSO-d_6), ppm: 4.89 (m, 2H, NH_2), 6.71-8.82 (m, 10H, =CH, Ar-H), 10.10 (s, -**NH**-CS), 9.45 (s, -**NH**-NH_2) 3.46 (s, 6H, -N(CH_3)$_2$). **^{13}C-NMR** (DMSO-d_6), ppm: 179.39 (C=S), 187.75 (C=O), 152.35 ((CH_3)$_2$N-**Ar**-CH=CH), 145.02 (Ar-**CH**=CH), 122.59 (Ar-**CH**=CH), 40.74 (CH_3), 131.13, 129.09, 128.93, 124.42, 123.83, 116.53, 112.22.

N-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)-2-phenylhydrazinecarbothioamide 3b. To a solution of 0.61 g (0.002 mol) 3-(4-(Dimethylamino)phenyl-1-(4-isothiocyanatophenyl)prop-2-en-1-one **1a** and 4 mL of benzene was added by droplet a solution of 0.21 g (0.002 mol) phenylhydrazine in 2 mL of benzene. The reacting mixture was let at room temperature for 1 hour and after heated at 40-45°C for 20 min. The end of the reaction was followed by chromatography by the consumption of isothiocyanatochalcone **1a**. The resulting crystals were filtrated and recrystallized from ethanol. Yield 0.73 g (88%) of thioamide **3b**, m.p. 219-221°C.
Elemental analysis and NMR data: Calculated of $C_{24}H_{24}N_4OS$ (**3b**), %: C-69.20, H-5.81, N-13.45. Found, %: C-69.32, H-5.61, N-13.65. **^{1}H-NMR** (DMSO-d_6), ppm: 6.73-8.10 (m, 15H, =CH, Ar-H), 10.12 (s, **NH**-CS), 9.96 (s, -**NH**-NH-Py), 8.16 (s, -NH-**NH**-Ar), 3.42 (s, 6H, -N(CH_3)$_2$). **^{13}C-NMR** (DMSO-d_6), ppm: 187.93 (C=O), 181.32 (C=S), 148.34 (-NH-NH-**Ar**), 143.71 (-C_6H_4-**NH**), 152.41 ((CH_3)$_2$N-**Ar**-CH=CH), 145.18 (Ar-**CH**=CH), 122.58 (Ar-**CH**=CH), 40.92 (CH_3), 134.65, 131.16, 129.39, 124.20, 116.56.
112.24.

2-Benzoyl-N-(4-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl)hydrazinecarbothioamide 3c. The mixture of 0.61 g (0.002 mol) 3-(4-(Dimethylamino)phenyl-1-(4-isothiocyanatophenyl)prop-2-en-1-one **1a**, 0.23 g (0.0021 mol) 2-hydrazinylpyridine and 6 mL of benzene was kept at room temperature for 1 hour and after heated at 40°C for 5 min. The total consumption of isothiocyanatochalcone **1a** was checked by chromatography. The resulting mixture was cooled and the resulting crystals were filtered out. Yield 0.76 g (92 %) of hydrazinecarbothioamide **3d** with m.p. 178-180°C, (from ethanol).
Elemental analysis and NMR data: Calculated of $C_{23}H_{23}N_5OS$ (**3d**), %: C-66.16, H-5.55, N-16.77. Found, %: C-66.18, H-5.52, N-16.79. **^{1}H-NMR** (DMSO-d_6), ppm: 6.68-8.16 (m, 13H, =CH, Ar-H, Py-H), 10.10 (s, **NH**-CS), 9.97 (s, **NH**-NH-Py), 8.65 (s, NH-**NH**-Py), 3.42 (s, 6H, -N(CH_3)$_2$). **^{13}C-NMR** (DMSO-d_6), ppm: 187.92 (C=O), 181.36 (C=S), 159.47 (-NH-NH- **Py**), 139.26 (-C_6H_4-**NH**), 152.42 ((CH_3)$_2$N-**Ar**-CH=CH), 145.14 (Ar-**CH**=CH), 122.59 (Ar-**CH**=CH), 131.16, 124.33, 116.58, 112.24, 107.94, 40.65 (CH_3).

N-(4-(3-(pyridin-2-yl)acryloyl)phenyl)hydrazinecarbothioamide 4a. The mixture of 0.53 g (0.002 mol) 1-(4-isothiocyanatophenyl)-3-(pyridine-2-yl)prop-2-en-1-one **1b**, 0.13 g (0.0025 mol) of hydrazine hydrate and 2 mL of benzene was stirred at room temperature for 2 hours until the total consumption of isothiocyanate **1b**. After the mixture was cooled down and the formed crystals were filtrated, washed with water and dried. Yield 0.38 g (64%) of carbothioamide **4a**, m.p. 169-171°C.
Elemental analysis and NMR data: Calculated of $C_{15}H_{14}N_4OS$ (**4a**), % C-60.38, H-4.73, N-18.78. Found, %: C-60.47, H-4.84, N-18.97. **^{1}H-NMR** (DMSO-d_6), ppm: 3.89 (m, 2H, NH_2), 7.36-8.70 (m, 10H, =CH, Ar-H), 10.50 (s, -**NH**-CS), 9.48 (s, -**NH**-NH_2). **^{13}C-NMR** (DMSO-d_6), ppm: 180.39 (C=S), 188.09 (C=O), 152.39 (**Py**-CH=CH), 144.96 (Ar-**CH**=CH), 122.59 (Ar-**CH**=CH), 137.42, 133.72, 126.58, 122.31.

2-(Pyridin-2-yl)-N-(4-(3-(pyridin-2-yl)acryloyl)phenyl)hydrazinecarbothioamide 4b. The solution of 0.53 g (0.002 mol) 1-(4-isothiocyanatophenyl)-3-(pyridine-2-yl)prop-2-en-1-one **1b**, 0.22 g (0.002 mol) 2-hydrazinylpyridine and 4mL of benzene was stirred at room temperature for 2 hours. After, the solution was cooled down and the resulting crystalline product was filtered and recrystallized from ethanol. Yield 0.64 g (85%) m.p. 198-200°C.
Elemental analysis and NMR data: Calculated of $C_{20}H_{17}N_5OS$ **4b**, %: C-63.98, H-4.56, N-18.65. Found, %: C-63.96, H-4.58, N-18.64. **^{1}H -NMR** (DMSO-d_6), ppm: 6.78-8.36 (m, 13H, =CH, Ar-H, Py-H), 10.12 (s, **NH**-CS), 9.91 (s, **NH**-NH-Py), 8.68 (s, NH-**NH**-Py). **^{13}C-NMR** (DMSO-d_6), ppm: 187.92 (C=O), 181.36 (C=S), 159.47 (-NH-NH- **Py**), 139.26 (-C_6H_4-**NH**), 154.42 (**Py**-CH=CH), 145.17 (Ar-**CH**=CH), 122.56 (Ar-**CH**=CH), 131.16, 124.33, 116.58, 112.24, 107.94.

N-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)-2-(pyridin-2-ylmethylene)hydrazinecarbothio-amide 5a. To the solution of 0.24 g (0.0023 mol) 2-formylpyridine, 0.1 g of CH_3COOH and 1 mL of dimethylformamide was

added under stirring droplet by droplet to a of 0.68 g (0.002 mol) N-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl) hydrazinecarbothioamide **3a** in 2 mL of dimethylformamide during 30 min at room temperature and after heated at 40°C. After, the reacting mixture was heated at 70°C for 3 hours (the consumption of carbothioamide **3a** was verified by chromatography), diluted with small amount of water and cooled. Yield 0.71 g (83%) of final product **5a** with m.p. 133-135°C, (from dimethylformamide).
Elemental analysis and NMR data: Calculated of $C_{24}H_{23}N_5OS$ **5a**, %: C-67.11, H-5.40, N-16.30. Found, %: C-67.10, H-5.42, N-16.33. **^{1}H-RMN** (DMSO-d_6), ppm: 6.74-8.61 (m, 14H, =CH, Ar-H, Py-H), 3.42 (s, 6H, -N(CH_3)$_2$), 10.46 (s, **NH**-CS), 12.24 (s, **NH**-N=CH). **^{13}C-NMR** (DMSO-d_6), ppm: 187.26 (C=S), 188.02 (C=O), 153.41 (**Py**-CH=N-NH-), 152.47 (-NH-N=**CH**-Py), 145.36 (Ar-**CH**=CH), 122.18(Ar-CH=**CH**-), 144.58 (-C_6H_4-**NH**), 40.92 (CH_3), 130.46, 128.87, 121.3.

Similar procedure was used for the synthesis of **N-(4-(3-(pyridin-2-yl)acryloyl)phenyl)-2-(pyridin-2-ylmethylene) hydrazinecarbothioamide 6a** with an yield of 77%, m.p. 192-194°C.
Elemental analysis and NMR data: Calculated of $C_{21}H_{17}N_5OS$ **6a**, %: C-65.10, H-4.42, N-18.08. Found, %: C-65.72, H-4.62, N-18.33. **^{1}H-RMN** (DMSO-d_6), ppm: 7.43-8.68 (m, 14H, =CH, Ar-H, Py-H), 3.42 (s, 6H, -N(CH_3)$_2$), 10.64 (s, **NH**-CS), 12.25 (s, **NH**-N=CH). **^{13}C-NMR** (DMSO-d_6), ppm: 188.94 (C=O), 176.37 (C=S), 153.08 (**Py**-CH=N-NH-), 150.48 (-NH-N=**CH**-Py), 145.47 (Ar-**CH**=CH), 125.57 (Ar-CH=**CH**-), 143.66 (-C_6H_4-**NH**), 134.15, 125.57, 122.33.

N-(4-(5-(4-(Dimethylamino)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)hydrazinecarbothioamide 7a. a) The mixture of 0.70 g (0.002 mol) 3-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)-1,1-dimethylthiourea **1**, 0.22 g (0.0044 mol) hydrazine hydrate and 5 mL of pyridine was let at room temperature for 24 hours and after heated at 90-95°C for 3 hours. The resulting crystals were filtered and recrystallized from methanol. Yield 0.54 g (77%) of thioamide **7a** with m.p. 173-174°C.
b) The mixture of 0.61 g (0,002 mol) 3-(4-(Dimethylamino)phenyl-1-(4-isothiocyanatophenyl)prop-2-en-1-one **1a**, 0.26 g (0.0025 mol) hydrazine hydrate and 5 mL of pyridine was left to stand at room temperature for 24 hours. The consumption of propenone **1a** was, verified by chromatography. After the reacting mixture, was heated at 90-95°C for 3 hours. The resulting product was isolated like in the case "a". Yield 0.58 g (82%) of thioamide **7a**, m.p. 172-174°C.
Elemental analysis and NMR data: Calculated of $C_{18}H_{22}N_6S$ **7a**, %: C-60.99, H-6.26, N-23.71. Found, %: C-61.07, H-6.21, N-23.74. **^{1}H-NMR** (DMSO-d_6), ppm: 4.73 (m, 2H, NH_2), 6.69-7.66 (m, 8H, =CH, Ar-H), 3.39 (m, 2H, CH_2), 10.39 (s, **NH**-CS), 9.89 (s, **NH**-NH_2). **^{13}C-NMR** (DMSO-d_6), ppm: 176.55 (C=S), 153.45 (**Ar**-CH-NH=N), 139.26 (-C_6H_4-**NH**), 63.86 (-CH_2-**C**(-C_6H_4-)=N), 40.95 (CH_3), 130.44, 129.46, 127.69, 125.54, 112.92, 63.82.
The **N-(4-(5-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)hydrazinecarbothioamide 8a** was obtained similarly from 1,1-dimethyl-3-(4-(3-(pyridin-2-il)-acryloyl)-phenyl)thiourea **2** and hydrazine hydrate with an yield of 77%, with m.p. 199-201°C (from ethanol).
Elemental analysis and NMR data: Calculated of $C_{15}H_{16}N_6S$ **8a**, %: C-57.67, H-5.16, N-26.90. Found, %: C-57.62, H-5.20, N-26.91. **^{1}H-NMR** (DMSO-d_6), ppm: 4.95 (m, 2H, NH_2), 7.27-8.58 (m, 8H, =CH, Ar-H, Py-H), 3.42 (m, 2H, CH_2), 9.19 (s, **NH**-CS), 8.53 (s, **NH**-NH_2). **^{13}C-NMR** (DMSO-d_6), ppm: 179.70 (C=S), 162.16 (**Py**-CH-NH=N), 150.05 (Py-**CH**(CH_2)NH-N=), 139.74 (-C_6H_4-**NH**), 64.89 (-CH_2-**C**(-C_6H_4-)=N), 149.44, 129.28, 125.85, 122.49, 64.89.

N-(4-(5-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-2-(pyridin-2-ylmethylene)-hydrazinecarbothioamide 9a. To a solution of 0.7g (0.002 mol) N-(4-(5-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)hydrazinecarbothioamide **7a** and 8 mL of CH_3COOH was added under stirring 0.22 g (0.002 mol) of 2-formylpyridine. The reacting mixture was let at room temperature for 24 hours and after heated at 70-80°C for 2 hours. After, the resulting mixture was neutralized with a solution of $NaHCO_3$. The resulting crystalline product was filtered and recrystallized from acetone. Yield 0.53 g (60%) of final product with m.p. 168-169°C.
Elemental analysis and NMR data: Calculated of $C_{22}H_{26}N_6S$ (**9a**), %: C-67.84, H-5.92, N-18.99. Found, %: C-67.65, H-5.75, N-18.41. **^{1}H- NMR** (DMSO-d_6), ppm: 6.52-8.63 (m, 13H, =CH, Ar-H, Py-H), 3.73 (m, 2H, CH_2), 10.34 (s, **NH**-CS), 12.18 (s, **NH**-N=CH), 3.62 (s, 6H, -N(CH_3)$_2$). **^{13}C-NMR** (DMSO-d_6), ppm: 178.26 (C=S), 156.26 (**Py**-CH=N-NH-), 152.73 (-NH-N=**CH**-Py), 52.03 (Ar-**CH**-(CH_2)NH-N=), 140.16 (-C_6H_4-**NH**), 64.95 (-CH_2-**C**(-C_6H_4-)=N), 149.98, 133.89, 129.20, 127.30, 122.90.

N-(4-(5-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-2-(pyridin-2-ylmethylene)- hydrazinecarbothioamide 10a. To the solution of 0.62g (0.002 mol) N-(4-(5-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl) hidrazinecarbothioamide **8a** and 8 mL of dimethylformamide was added under stirring 0.22 g (0.002 mol) of 2-formylpyridine, previously dissolved in 1 mL of ethanol. The reacting mixture was heated at 70-80°C for 2 hours, after diluted with water and cooled to room temperature. The resulting crystalline product was filtered and recrystallized from ethanol. Yield 0.48 g (63%) of final product **10a** with m.p. 140-142°C.
Elemental analysis and NMR data: Calculated of $C_{21}H_{19}N_7S$ (**10a**), %: C-62.82, H-4.77, N-24.42. Found, %: C-62.85,

H-4.75, N-24.41. **^{1}H-NMR** (DMSO-d_6), ppm: 6.10-8.67 (m, 13H, =CH, Ar-H, Py-H), 3.33 (m, 2H, CH_2), 10.42 (s, **NH**-CS), 12.22 (s, **NH**-N=CH). **^{13}C-NMR** (DMSO-d_6), ppm: 176.66 (C=S), 158.18 (**Py**-CH=N-NH-), 152.16 (-NH-N=**CH**-Py), 152.07 (Py-**CH**-(CH_2)-NH-N=), 139.30 (-C_6H_4-**NH**), 64.95 (-CH_2-**C**(-C_6H_4-)=N), 143.98, 138.89, 129.28, 127.79.

Cytotoxicity Assay

Cell culture. Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD, USA) were routinely grown in suspension in 90% RPMI-1640 (Sigma, Saint Louis, USA) containing L- glutamine (2 mM), antibiotics (100 IU penicillin/mL, 100 mg streptomycin/mL) and supplemented with 10% (v/v) foetal bovine serum (FBS), in a 5% CO_2 humidified atmosphere at 37°C. Cells were currently maintained twice a week by diluting the cells in RPMI 1640 medium containing 10% FBS.

Cell proliferation assay. The cell proliferation assay for compounds and ligands was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl) 2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Cell Titer 96 Aqueous, Promega, USA), which allowed us to measure the number of viable cells. In brief, triplicate cultures of 10,000 cells in a total of 100 mL medium in 96-well microtiter plates (Becton Dickinson and Company, Lincoln Park, NJ, USA) were incubated at 37°C, 5% CO_2. All compounds were dissolved in ethanol to prepare the stock solution of 1 J 1022 M. These compounds and doxorubicin (Novapharm, Toronto, Canada) which was used as a positive control were diluted at multiple concentrations (1 and 10 μM) with culture media and added to each well and incubated for 3 days. Following each treatment, MTS (20 μL) was added to each well and the mixture was incubated for 4 hours. MTS is, converted to water-soluble colored formazan by dehydrogenase enzymes present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA).

References

1. Dzurilla, M.; Kristian, P. The synthesis infrared and ultraviolet absorption spectra of 4-substituted 3′- and 3′-isothiocyanatochalcones. Collection of Czechoslovak Chemical Communications, 1970, 35, pp. 417-429.
2. Barbă, N.; Luchiţa, G.; Barbă, A.; Vieru, R. Process for isothiocyanatochalcones preparation, Patent for invention MD 2985, 2004 (in Romanian).
3. Kessler, M.A.; Meinitzer, A.; Wolfbeis, O.S. Albumin Blue 580 fluorescence assay for Albumin. Analytical Biochemestry, 1997, 248, pp.180-182.
4. Popusoi, A.; Barba, N.; Dragalina, G.; Culeac, I.; Robu, Ş. Synthesis and study of materials of copolymers of luminophores 4-aminostiren grafted with isothiocyantochalcones. The XXXI-st Romanian Chemical Conference, Romania, 2010, p. 141 (in Romanian).
5. Pelkis, P.S.; Pereteajco M.Z. Synthesis of substituted 1,4-diphenylthiosemicarbazides. Russian Journal of General Chemistry, 1961, XXXI(11), pp. 3726-3728 (in Russian).
6. Yousef, T.A.; Badria, F.A.; Ghazy, S.E. In vitro and in vivo antitumor activity of some synthesized 4-(2-pyridyl)-3-Thiosemicarbazides derivatives. International Journal of Medicine and Medical Sciences, 2011, 3, pp. 37–46.
7. Gutu, Ia.; Boi, L.V.; Barba, N. Process for 4-aryl and 1,4-diarylthiosemicarbazides preparation. Inventor's certificate USSR 1643533, 15, 1991 (in Russian).
8. Suvitha, S.; Siddig, I. A. Synthesis of Chalcones with Anticancer Activities. Molecules, 2012, 17, pp. 6179-6195.
9. Hu, K.; Yang, Z.H.; Pan, S.S.; Xu, H.J.; Ren, J. Synthesis and antitumor activity of liquiritigenin thiosemicarbazone derivatives. European Journal of Medicinal Chemistry, 2010, 45, pp. 3453-3458.
10. Hong-Jia, Z.; Yong, Q.; Di-Di, Z.; Xu-Guang, Y. Synthesis, molecular modeling and biological evaluation of chalcone thiosemicarbazide derivatives as novel anticancer agents. European Journal of Medicinal Chemistry, 2011, 46, pp. 4702-4708.
11. Ozan, R.; Zuhal, O.; Unsal, C. Synthesis of and Pharmacological Studies on The Antidepressant and Anticonvulsant Activites of Some 1,3,5-trisustituted pyrazolines. Arzneim-Forsch. Drug Research, 2005, 55(8), pp. 431-436.
12. Robinson, T.P.; Hubbard, R.B.; Ehlers, T.J.; Arbiser, J.L.; Goldsmithd, D.J.; Bowena, J.P. Synthesis and biological evaluation of aromatic enones related to curcumin. Bioorganic Medicinal Chemistry, 2005, 13, pp. 4007-4013.
13. Gulea, A.; Poirier, D.; Roy, J.; Stavila, V.; Bulimestru, I.; Ţapcov, V.; Bârcă, M.; Popovsch, L. In vitro antileukemia, antibacterial and antifungal activities of some 3d metal complexes: Chemical synthesis and structure-activity relationships. Journal of Enzyme Inhibition and Medicinal Chemistry, 2008, 23, pp. 806-818.