SYNTHESIS, STRUCTURE AND *IN VITRO* ANTIPROLIFERATIVE ACTIVITY OF SOME HYDRAZONES AND THEIR COPPER COMPLEXES

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Rezumat

Lucrarea conţine date despre sinteza, caracterizarea şi evaluarea *in vitro* a activităţii biologice a hidrazonelor în baza 2-hidrazinobenzotiazolului şi a 4 compuşi complecşi obţinuţi în baza 2-[2-(Piridin-2-ilmetilene)hidrazinil]-benzo[d]tiazolului şi 2-[2-(1-

Piridin-2-il)etilidene)hidrazinil]benzo[d]tiazolului dihidrat. Compoziţia compuşilor sintetizaţi a fost determinată cu ajutorul spectroscopiei ^{1}H, ^{13}C RMN. Toţi compuşii obţinuţi au fost testaţi ca inhibitori ai proliferării celulelor de leucemie umană HL-60. A fost stabilit că hidrazonele în baza 2-piridin aldehidei, aldehidei salicilice şi compuşii complecşi ai cuprului în baza 2-[2-(Piridin-2-ilmetilene)hidrazinil]-benzo[d]tiazolului şi 2-[2-(1-Piridin-2-il)etilidene)hidrazinil]benzo[d]tiazolului dihidrat sunt cei mai activi agenţi antiproliferativi obţinuţi în acest studiu. Unii din compuşi au fost testaţi ca inhibitori de proliferare a celulelor HepG2, MCF-7 şi LNCaP. Au fost calculate constantele de stabilitate a compuşilor complecşi în baza 2-[2-(Piridin-2-ilmetilene)hidrazinil]-benzo[d] tiazolului şi 2-[2-(1-Piridin-2-il)etilidene)hidrazinil]benzo[d]tiazolului dihidrat.

Cuvinte cheie: 2-hidrazinobenzotiazol – antiproliferative – leucemie - constanta de stabilitate.

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Introduction

Heterocyclic compounds containing nitrogen and sulphur possess potential pharmacological activities. Benzothiazoles are bicyclic ring systems which have been the subject of great interest because of important biological activities. Benzothiazole moiety possesses diverse type of biological activities: antifungal [10], antibacterial [12], antihelminthic [11], antimalarial [8], analgesic [1], anti-inflammatory [16], anticancer [3] and various central nervous system (CNS) activities.

The interest in the study of hydrazones possessing potential donor sites has been intensively increasing because of their coordination capability, their pharmacological activity and their uses in analytical chemistry as metal extracting agents. [4,7].It has recently been shown that the metal complexes are more potent and less toxic in many cases as compared to the parent compound. Therefore, we have started a project directed towards the synthesis of different classes of compounds that can act as molecular inhibitors of cancer cells proliferation [5,6,13]

In continuation of this approach, the present paper describes the synthesis, characterization and *in vitro* biological evaluation of some benzothiazoles, Schiff bases and their copper complexes. The composition and the structure of the synthesized substances have been determined by ^{1}H and ^{13}C NMR spectroscopy. All substances were tested as inhibitors of human leukaemia (HL-60) cell growth. Most active substance were tested as inhibitors of human hepatoma (HepG2) cell, prostate cancer cell line (LNCaP) and breast cancer (MCF-7) cells.

Materials and methods

*General***.** All reagents and chemicals have been obtained from commercially available sources, have been of analytical - or reagent-grade purity and have been used without further purification. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra have been recorded at room temperature with a Bruker DRX 400 spectrometer. All chemical shifts (^{1}H ,^{13}C) are given in ppm versus $SiMe_4$ using DMSO-d_6 as solvent. The complexes were analysed for their metal contents by EDTA titration [14]. Magnetic measurements were carried out on solid complexes using the Gouy's method [15]. The

stability constants for the synthesized compounds was calculated by Benesi-Hildebrand method [2].

Antileukaemia bioassay. Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD, USA) were routinely grown in suspension in 90% RPMI-1640 (Sigma, Saint Louis, MO, USA) containing L-glutamine (2 nM) and antibiotics (100 IU penicillin/mL, 100 µg streptomycin/mL), supplemented with 10% (v/v) foetal bovine serum (FBS), in a 5% CO_2 humidified atmosphere at 37 °C. Cell were currently maintained twice a week by diluting the cells in RPMI- 1640 medium containing 10% FBS. The cell proliferation assay was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)2-(4-sulfophenyl)-2H-tetrazolium (MTS) (Cell Titer 96 Aqueous, Promega, Madison, WI, USA), which allowed us to measure the number of viable cells. In a nutshell, triplicate cultures of 10,000 cells in a total of 100 µL medium in 96-well microtiter plates (Becton–Dickinson Company, Lincoln Park, NJ, USA) were incubated at 37 °C, 5% CO_2, for 24 hours. Compounds were dissolved in DMSO (Dimethyl sulfoxide) to prepare the stock solution of 1×10^{-2} M. Reference compounds used were: Doxorubicin (Novapharm, Toronto, Canada), CMT-61, CMT67, Lot 25B, PC-37. All the compounds were diluted at two concentrations (1µM and 10µM) with culture media, added to each well, and incubated for 3 days. Following each treatment, MTS (20 µL) was added to each well and incubated for 4 h. 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, USA).

Anticarcinoma bioassay. Human hepatoma HepG2 cells and human epithelial colorectal adenocarcinoma Caco-2 cells were grown in DMEM containing 100 µmol/L nonessential amino acids, 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum (FBS) or lipoprotein-deficient serum (LPDS), in a humidified atmosphere (5% CO2, 37°C). Typically, 106 cells per well were seeded in 6-well plates and grown to 70% to 80% confluence. The mRNA of PCSK9 and Nieman-Pick C1-like-1 protein (NPC1L1, the target of ezetimibe for inhibiting cholesterol absorption) were measured by quantitative RT-PCR. Cell viability was determined by the MTT assay. Approximately 2,000 HepG2 cells were plated in each well of a 96-well plate. After overnight incubation, the cells were treated with dsRNAs for 48-72 h and the concentration of dsWT1-319 arranged from 2 to 50 nM. At the various times following treatment, the medium was removed and MTT (20 µl of 5 mg/mL) was added to each well and incubated at 37°C for 4 h. The plates were spun, and the purple colored precipitates of formazan were dissolved in 150 µl of dimethyl sulfoxide. Absorbance was measured at 490 nm using the MRX II absorbance reader (DYNEX Technologies, Chantilly, Virginia, USA). The reduction in viability of in dsWT1 or dsControl treated HepG2 cells were expressed as a percentage compared to mock cells. Mock cells were considered to be 100% viable.

Antiprostate cancer bioassay. Prostate cancer cell line LNCaP, was obtained from the American Type Culture Collection (ATCC) and maintained in a 75 cm^2 culture flask at 37^0C under 5% CO_2 humidified atmosphere. The cells (passages between 25 and 44) were grown in RPMI medium supplemented with 10% fetal bovine serum (FBS),l-glutamine (2mM), penicillin (100 IU/mL) and streptomycin (100 µg/mL).

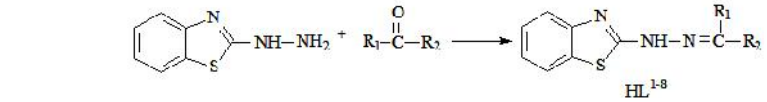
Quantification of cell growth was determined by using CellTitter 96® AQueous Solution Cell Proliferation Assay (Promega, Nepean, ON, Canada) following the manufacturer's instructions. . LNCaP cells were seeded in 96-well plates, each well containing about 5×103 cells, and transfected using Lipofectamine 2000 with different amount of 20 bp inhibitory decoy (1 µg/ml, 2 µg/ml or 4 µg/ml in each well). Ten microliters of MTT (0.5 mg/ml) was added 24, 48 and 72 h after transfection with the decoy. After incubation at 37 °C for 4 h, the supernatant was aspirated, and 100 µl DMSO was added to each well. Absorbance at 570 nm was measured by using a microplate reader. Assays were performed in quadruplicate.

Antibreast cancer bioassay. Breaast cancer cell line MCF-7, was obtained from the American Type Culture Collection (ATCC, Manassas,VA). MCF-7 cells were maintained in DME low-glucose medium supplemented with 1 nM β-estradiol. The cells were cultured in the presence of 10% FBS at 37 ^{0}C in a humidified atmosphere of 95% air and 5% CO_2. When indicated, FBS was treated overnight at 4^{0}C with 2% dextran-coated charcoal to remove the remaining steroids present in the serum. Cells were trypsinized for the passage into the well plate and plated at 10,000 cells/well in 100 µL of medium in 96-well plates. Cells were allowed to adhere to the surface of well plates. After 24 h, medium was removed and 100 µL of drug solutions were added into the wells. 100 µL of fresh medium without cells was added as control. 4 wells were used for each concentration of drug solution, while 4 wells were reserved for cell culture control, which contained the corresponding amount of DMSO. The total drug exposure was 48 h. After 48 h, contents of the well were removed and 20 µL of MTT solution (5 mg in 1 mL of phosphate buffer saline) was added to each well. Incubation at 37 °C for 4 h allowed reduction of MTT by mitochondrial dehydrogenase to an insoluble formazan product. Well contents were removed and the formazan product was solubilised by addition of 100 µL DMSO. The purple colour was produced. Absorbance of each well was read on Tenac 200 plate reader at 570 nm

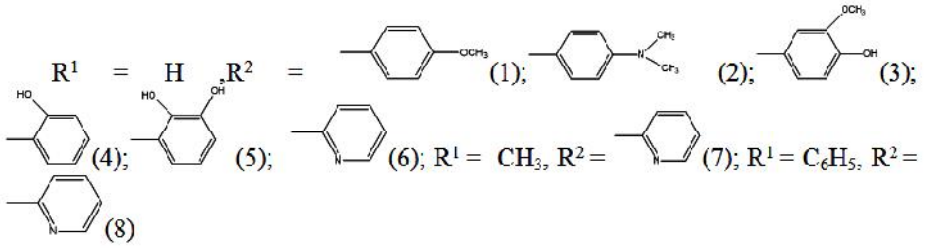
Synthesis of hydrazones HL^1-HL^8

The synthesis of hydrazones has been performed according to the published methods [9] (Scheme 1), with some particular modifications:

Scheme 1



HL$^{1-8}$



Synthesis of hydrazones HL^1-HL^8

2-(2-(4-Methoxybenzylidene)hydrazinyl)benzo[d]thiazole (HL^1): 1,36 g (0,01mol) 2-hydrazinobenzothiazole was added to a hot solution of 1,36 g (0,01 mol) 4-methoxybenzaldehyde in 7 mL ethanol and 4-5 drops of dimethylsulfoxide. The

resulting mixture was refluxed for 1.5 h. Then, the solution was allowed to reach ambient temperature and cooled to 0^0C overnight. The crude product was filtered, dried and recrystalized from ethanol. Yield, 2,67 g (94,5%) ; m.p. 195-196^0 C. ^{1}H-NMR (DMSO-d_6): 12.1 (s, 1H, NH); 8.1 (s, 1H, N=CH); 7.75, 7.43, 7.29,7.11 (m, 4H, benzothiazole); 7.02, 7.02, 7.64, 7.64 (m, 4H, benzene); 4.2(s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6): 55.77 (O-CH_3); 114.85, 114.85, 128.58, 128.58 (benzene); 121.89, 121.93,126.37, 127.47 and 129.65 (benzothiazole); 144.56 (N=CH); 160.98 (C-O);167.38 (C-NH).

4-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl) – N,N- dimethylaniline (HL^2) : 1,65 g (0,01 mol) 2-hydrazinobenzothiazole was added to a hot solution of 1,49 g (0,01 mol) 4-dimethylaminobenzaldehyde in 7 mL ethanol and 4-5 drops of dimethylsulfoxide. The resulting mixture was refluxed for 1.5 h. Then, the solution was allowed to reach ambient temperature and cooled to 0^0C overnight. The crude product was filtered, dried and recrystalized from ethanol. Yield, 2.47g (83,3 %); p.t. 242-243^{0}C . ^{1}H-NMR (DMSO-d_6): 11.93 (s, 1H, NH); 8.01 (s, 1H, N=CH); 7.75, 7.52, 7.50, 7.41 (m, 4H, benzothiazole); 7.29, 7.29, 7.09, 6.76 (m, 4H,benzene); 3,01(s, 3H, CH_3); 2,98(s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6): 40.6 (N-CH_3); 111.52, 112.38, 121.64, 121.87 (benzene); 117.80, 122.25, 126.30, 128.34, 133.05 (benzothiazole); 129,65 (CH-C); 151,46 (N=CH); 145.46 (C-N); 167,12 (C-NH).

4-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)-2-metoxyphenol (HL^3): 1,65 g (0,01 mol) 2-hydrazinobenzothiazole was added to a hot solution of 1,52 g (0,01 mol) 4-hydroxy-3-methoxybenzaldehyde in 7 mL ethanol and 4-5 drops of dimethylsulfoxide. The resulting mixture was refluxed for 1.5 h. Then, the solution was allowed to reach ambient temperature and cooled to 0^0C overnight. The crude product was filtered, dried and recrystalized from ethanol. Yield, 2.84g (95%) ; m.p.197-198^{0}C.^{1}H-NMR (DMSO-d_6): 12.1(s, 1H, OH); 9.5 (s, 1H, NH); 8.05 (s, 1H, N=CH); 7.75, 7.43, 7.29,7.29 (m, 4H, benzothiazole); 7.1, 7.1, 6,87, (m, 3H,benzene); 3,84 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6): 56.02 (O-CH_3); 109.82, 116.11, 121.92 (benzene); 117.8, 121.43, 121.81,129.63, 150.43 (benzothiazole); 145.1 (CH-C); 148.46 (N-CH); 149.05 (C=O); 167.31(C-OH); 172.46 (C-NH).

2-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)phenol (HL^4) : 1,65 g (0,01 mol) 2-hydrazinobenzothiazole was added to a hot solution of 1,22 g (0,01 mol) salicylaldehyde in 7 mL ethanol and 4-5 drops of dimethylsulfoxide. The resulting mixture was refluxed for 2 h. Then, the solution was allowed to reach ambient temperature and cooled to 0^0C overnight. The crude product was filtered, dried and recrystalized from ethanol. Yield, 2.59g (96,2%); m.p. 250^{0}C.^{1}H NMR (DMSO-d_6): 12.19 (s, 1H, OH); 10.48 (s 1H, NH); 8.46 (s, 1H, N=CH); 7.76, 7.63, 7.38, 7.32 (m, 4H, benzothiazole); 7.30, 7.1,6.93, 6.92 (m, 4H,benzene).

3-((2-(Benzo[d]thiazol-2yl)hydrazono)methyl)benzene-1,2-diol (HL^5): 1,65 g (0,01 mol) 2-hydrazinobenzothiazole was added to a hot solution of 1,38 g (0,01 mol) 2,3-dihydroxybenzaldehide in 7 mL ethanol and 4-5 drops of dimethylsulfoxide. The resulting mixture was refluxed for 1 h. Then, the solution was allowed to reach ambient temperature and cooled to 0^0C overnight. The crude product was filtered, dried and recrystalized from ethanol. Yield, 2.59g (90,9%) ; m.p. 255^{0}C. ^{1}H-NMR (DMSO-d_6): 12.17(s, 1H, OH); 9.99 (s, 1H, OH); 9.38 (s, 1H, NH); 8.45 (s, 1H, N=CH); 7.75, 7.37, 7.28,7.13 (m, 4H, benzothiazole); 7.06, 6.85, 6,70 (m, 3H, benzene). ^{13}C-NMR

(DMSO-d_6): 117.26, 117.26, 119.01, (benzene); 119.80, 119.80, 120.41,120.41,122.04 (benzothiazole); 122.33 (CH-C); 148.46 (N=C); 145.89 (C-OH); 146.03(C-OH); 166.74 (C-NH).

2-[2-(Pyridin-2-ylmethylene)hydrazinyl]-benzo[d]thiazole (HL^6) : 1,65 g (0,01 mol) 2-hydrazinobenzothiazole was added to a hot solution of 1,07 g (0,01 mol) 2-pyridinecarboxaldehyde in 7 mL ethanol and 4-5 drops of dimethylsulfoxide. The resulting mixture was refluxed for 2 h. Then, the solution was allowed to reach ambient temperature and cooled to 0^0C overnight. The crude product was filtered, dried and recrystalized from ethanol. Yield, 2.04g (80%) ; m.p. 230-231^{0}C.^{1}H-NMR (DMSO-d_6): 12.53 (s, 1H, NH); 8.6 (s, 1H, N=CH); 8.14, 7.9, 7.86 ,7,80 (m,4H, pyridine); 7.60, 7.38, 7.35 , 7.15 (m, 4H, benzothiazole).

2-[2-(1-Pyridin-2-yl)ethylidene)hydrazinyl]benzo[d]thiazole dihydrate (HL^7): 1,65 g (0,01 mol) 2-hydrazinobenzothiazole was added to a hot solution of 1,21 g (0,01 mol) 2- acetylpyridine in 7 mL ethanol and 4-5 drops of dimethylsulfoxide. The resulting mixture was refluxed for 4 h. Then, the solution was allowed to reach ambient temperature and cooled to 0^0C overnight. The crude product was filtered, dried and recrystalized from ethanol. Yield, 1.51g (52,8 %) ; m.p.143-145^{0}C.^{1}H-NMR (DMSO-d_6): 11.87 (s, 1H, NH); 8.6 (s, 1H, N=CH); 8.1, 7.8,7.8 , 7.76 (m,4H, pyridine); 7.40, 7.38, 7.32 , 7.15 (m, 4H, benzothiazole); 2.43 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6):13.2 (CH_3); 120.3, 120.3, 122.24, 122.24, 136.92 (benzothiazole); 124.06, 124.06,126.5, 126.5 (pyridine); 149.11(N=C); 155.58 (C-C); 168.71 (C-NH).

2-(2-(phenyl(pyridin-2-yl)methylene)hydrazinyl)benzo[d]thiazole (HL^8) : 1,65 g (0,01 mol) 2-hydrazinobenzothiazole was added to a hot solution of 1,83 g (0,01 mol) 2-benzoilpyridine in 7 mL ethanol and 4-5 drops of dimethylsulfoxide. The resulting mixture was refluxed for 4 h. Then, the solution was allowed to reach ambient temperature and cooled to 0^0C overnight. The crude product was filtered, dried and recrystalized from ethanol. Yield, 2.32g (70,5%) ; m.p.100-101^{0}C.^{1}H RMN (DMSO-d_6): 12.1(s, 1H, NH); 8.47, 8.18, 7.92,7.74 (m, 4H, pyridine); 7.53, 7.53, 7.26, 7.26 (m, 4H,benzene); 7.53, 7.53, 7.29,7.29 (m, 4H, benzothiazole). ^{13}C RMN (DMSO-d_6): 121.7, 122.2, 123.85,135.28, 149.55 (benzothiazole); 128.46, 128.89, 129.49, 129.96, 136.91, 137.57 (benzene); 123.8, 125.8,137.66, 149.06 (pyridine); 156.43 (C-C); 168.33 (N=C); 169.26 (C-NH).

Synthesis of copper complexes 1-4

[Cu(HL^6)Cl_2] (1) To a solution of Cu(II) chloride (0.005 mol) in ethanol (15 mL), heated (50-55^{0}C) and mixed continuously with a magnetic agitator , was added a solution of 2-[2-(Pyridin-2-ylmethylene)hydrazinyl]-benzo[d]thiazole (0.005 mol) in ethanol (10 mL) and the mixture was heated for 30-40 min. After cooling, the small brown crystals formed from the reaction mixture were filtered washed with ethanol and dried in air. Yield: 85%.

[Cu(HL^6)(NO_3)$_2$](2) To a solution of Cu(II) nitrate trihydrate (0.005 mol) in ethanol (15 mL), heated (50-55^{0}C) and mixed continuously with a magnetic agitator , was added a solution of 2-[2-(Pyridin-2-ylmethylene)hydrazinyl]-benzo[d]thiazole (0.005 mol) in ethanol (10 mL) and the mixture was heated for 30-40 min. After cooling, the small brown crystals formed from the reaction mixture were filtered washed with ethanol and dried in air. Yield: 79%.

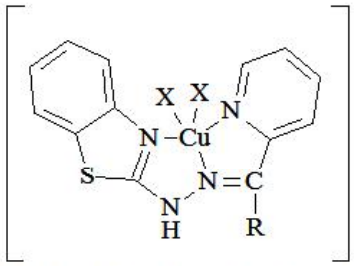
[Cu(HL^7) Cl$_2$] (3) To a solution of Cu(II) chloride (0.005 mol) in ethanol (15 mL), heated (50-55^{0}C) and mixed continuously with a magnetic agitator , was added a solution of 2-[2-(1-Pyridin-2-yl)ethylidene)hydrazinyl]benzo[d]thiazole dihydrate (0.005 mol) in ethanol (10 mL) and the mixture was heated for 30-40 min. After cooling, the small brown crystals formed from the reaction mixture were filtered washed with ethanol and dried in air. Yield: 80%.

[Cu(HL^7) (NO$_3$)$_2$](4) To a solution of Cu(II) nitrate trihydrate (0.005 mol) in ethanol (15 mL), heated (50-55^{0}C) and mixed continuously with a magnetic agitator , was added a solution of 2-[2-(1-Pyridin-2-yl)ethylidene)hydrazinyl]benzo[d]thiazole dihydrate (0.005 mol) in ethanol (10 mL) and the mixture was heated for 30-40 min. After cooling, the small brown crystals formed from the reaction mixture were filtered washed with ethanol and dried in air. Yield: 80%.

Table 1. Physical and analytical data of the copper complexes

Copper complexes	Mr g/mol	μ eff (B.M.)	Exp./teor. (%)	η (%)
[Cu(HL^6) Cl$_2$]	388	2.19	16.38(16,45)	85
[Cu(HL^6)(NO$_3$)$_2$]	442	1.91	14.80(14.88)	79
[Cu(HL^7) Cl$_2$]	402	1.76	15.80(15.88)	80
[Cu(HL^7)(NO$_3$)$_2$]	456	1.78	14.025(14.03)	80

Schematic srtucture of complexes **1-4** is presented in Figure 1.



R = H, X = Cl (1); R = H, X = NO$_3$ (2); R = CH$_3$, X = Cl (3); R = CH$_3$, X = NO$_3$(4)

Fig. 1 . Structure of copper complexes 1-4 .

Results and discussions.

Antiproliferative activity. All substances were tested as inhibitors of HL-60 cells proliferation. These human promyelocytic leukaemia cells were incubated for three days in the presence of synthetic compounds an the number of viable cells was measured using the MTS assay (Fig. 2).

The results are expressed as the percentage of cell growth inhibition. As it can be concluded from the date above substituted hydrazones 1-3 do not show antiproliferative activity at any concentration. The highest antiproliferative activity show the hydrazones in base of the 2-pyridinecarboxaldehyde , 2-acetylpyridine, 2-benzoylpyridine and less activity in the salicylaldehyde (HL^4)**.** The antiproliferative activity of hydrazones in base of salicylaldehyde (HL^4.) depended by number and position of OH groups in the composition of radical R^2. The introduction of a second OH group in the benzene ring results in increased activity of 4-9 times. Therefore, it can be inferred that the

antiproliferative activity of the compounds HL^6-HL^8 is influenced by the nature of R^1 and its grows in the following order: H< CH_3<C_6H_5. The complexes 1-4 show a important antiproliferative activity at all concentrations.

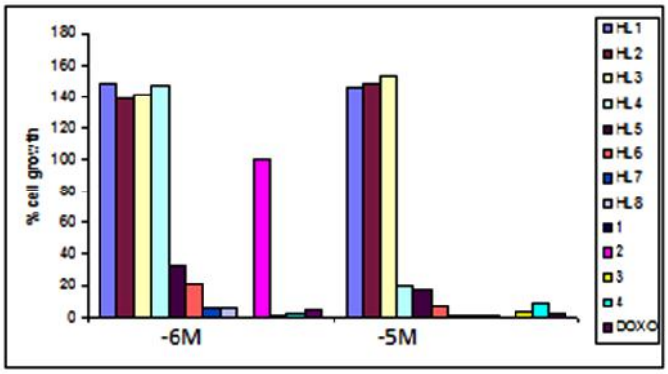


Fig. 2.. The inhibition effect on HL-60 cancer cells grown of hydrazones and their complexes.

Table 2. Antiproliferative activity of some compounds against human myeloid leukaemia HL-60), prostate cancer cells LNCaP, breast cancer cells MCF-7, human hepatoma cancer cells HepG2.

Inhibitor	HL-60			LNCaP			MCF-7			HepG-2		
	10 µM	1,0 µM	0,1 µM	10 µM	1,0 µM	0,1 µM	10 µM	1,0 µM	0,1 µM	10 µM	1,0 µM	0,1 µM
HL^8	99	95	72	2.0	6.6	0	42.3	48.2	31.8			
HL^7	99	95	92	0	0	12.7	3.8	13.2	0	86.4	11.2	0
$[Cu(HL^7)Cl_2]$	100	100	100	77.2	100	15.6	100	100	44.7			
$[Cu(HL^7)(NO_3)_2]$	90	100	100	98.0	15.5	4.7	99.0	90.5	45.1			
Doxorubicin	95	92	16	96.1	76	15	50.4	4.3	0.6	92.9	20.9	0

SEM < ± 4% of a single experiment in triplicate

Some of substancs were tested on other type of antiproliferation activity (Table 2). The copper complexes with 2-[2-(1-Pyridin-2-yl)ethylidene)hydrazinyl]benzo [d] thiazole dehydrate show the highest activity at human myeloid leukaemia HL-60 prostate cancer (LNCaP) and breast cancer (MCF-7) at all three concentrations. The 2-[2-(1-Pyridin-2-yl) ethylidene) hydrazinyl]benzo[d]thiazole dihydrate show the low activity at prostate cancer (LNCaP) and breast cancer (MCF-7).

Magnetic measurements of the coordination compounds showed that all the complexes are paramagnetic, and judging from the values of the effective magnetic moments are monomeric structure.(Table 1)

Copper as an essential element is present in many metalloproteins and enzymes and it is playing an important role in electron transfer reactions of many cellular processes. However, excessive quantities of copper can be very toxic and can result in several diseases. Therefore, organic compounds that can interfere with copper homeostasis may find therapeutic application in the treatment of copper induced diseases. It has been found by means of izomolare series method that 2-[2-(Pyridin-2-ylmethylene) hydrazinyl]-benzo[d]thiazole (HL^6) and 2-[2-(1-Pyridin-2-yl)ethylidene) hydrazinyl] benzo[d]thiazole dihydrate (HL^7) form with Cu(II), Ni(III), Co(II) complexes with composition 1 : 1. In order to calculate the stability constants for the synthesized

compounds, Benesi-Hildebrand method has been used. Stability constants have been calculated by the following equation

$$b = \frac{[CuHl^{6,7}]}{([C_{Cu}] - [CuHL^{6,7}])^2}$$

Table 3 . The stability constants of copper complexes in aqua solutions.

ligand/salt	$CuCl_2*2H_2O$	$Cu(NO_3)_2*3H_2O$
HL[6]	lg β=5,319	lg β= 5,59
HL[7]	lgβ= 5,149	lgβ=6,705

In case of the complexes formed by the copper chloride and nitrate with HL^6, almost identical values for the stability constants have been obtained. However, if the same salts are used with HL^7, the complex formed from copper nitrate has a much greater stability constant . This is due to the fact that all of the defined constants are apparent and are pH-dependent.

Conclusions

Eight hydrazones on the basis of 2-hydrazinobenzothiazole and four copper complexes in base of 2-[2-(Pyridin-2-ylmethylene)hydrazinyl]-benzo[d]thiazole (HL^6) and 2-[2-(1-Pyridin-2-yl)ethylidene)hydrazinyl]benzo[d]thiazole dihydrate (HL^7) have been obtained.The composition and the structure of the synthesized compounds have been defined by means of ^1H, ^{13}C NMR. The obtained hydrazones and copper complexes inhibits the growth of HL-60 cells of human leukemia myeloid within the concentrations range 10^{-5}-10^{-6} mol/l. The highest activity showed the compounds obtained on the basis of 2-pyridinecarboxaldehyde, 2-acetylpyridine, 2-benzoylpyridine and less activity on the basis of salicylaldehyde. (HL^4)The properties of compounds represent an interest for medicine in terms of expanding of an arsenal of inhibitors of human leukemia HL-60 myeloid. Therefore, the most active compounds 1-4 should be further studied as potential alternatives to traditional antileukaemia medicines.The hydrazones manifest comparable activity with such drugs as doxorubicin (Doxo) used in medicine for the leukemia treatment. The metal complexes in base of 2-[2-(1-Pyridin-2-yl)ethylidene) hydrazinyl]benzo[d] thiazole dihydrate show the highest activity at human myeloid leukaemia HL-60 prostate cancer (LNCaP) and breast cancer (MCF-7) at all three concentrations. The 2-[2-(1-Pyridin-2-yl)ethylidene) hydrazinyl]benzo[d]thiazole dihydrate show the low activity at prostate cancer (LNCaP) and breast cancer (MCF-7). All of the defined constants are apparent and are pH-dependent.. Magnetic measurements of the coordination compounds showed that all the complexes are paramagnetic, and have monomeric structure.

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